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Physiological Zoölogy

JULY, 1934

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Physiological Zoölogy

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COLOR CHANGES IN TRANSPLANTED TADPOLE SKIN[1]

(Two figures)

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RECENT investigations indicate that the color changes of amphibians depend largely on the presence of the eyes, the chief organs for light reception which probably induce either through direct nerve connection or through some hormone produced in the eye (Kropp, 1929; Perkins and Kropp, 1932), the secretion of chromatophore activating substances by endocrine glands (pituitary and adrenals[?], Hogben, 1924; Parker, 1930), these hormones being distributed to the pigment cells by the blood (see Parker, 1930; 1932 for complete bibliography). In the following note are reported the activities of the melanophores in transplanted tadpole skin, results which are further evidence for a humoral control of the chromatic changes of amphibians. The species used for these observations was *Rana areolata*.[2] In some experiments animals in which no hind legs had yet appeared were used. These are called young tadpoles; the others (with hind legs) are called old tadpoles.

In 1922 Cole noted that for *R. clamitans* and *R. catesbeiana* either expansion or contraction of the melanophores required about 36 hours for completion. In a series of preliminary observations I found

[1] This investigation was aided by a grant from the American Association for the Advancement of Science, 1931.

[2] The species was identified by A. H. Wright, of Cornell University, to whom I wish to extend my thanks.

that the animals used assumed the maximum contraction or expansion state in from 24 to 36 hours and that in moribund individuals the melanophores became reticulate and completely unresponsive to environmental color changes.

Since in the experiments given below chloretone was to be used for narcotization, studies on the effect of chloretone were made. According to Cole (1922), a 0.1 per cent chloretone solution caused maximum expansion of the melanophores in 30 minutes when the animal was placed in diffused daylight. Kropp (1927) states that "when tadpoles are narcotized with chloretone their melanophores expand completely"; further, that upon recovery from the anesthesia the "melanophores show every evidence of being normal. They respond promptly to light and dark backgrounds . . . . and show no evidence of injury or paralysis." Unfortunately, Kropp does not give the concentration of his solution so that one cannot compare his results with the work of Cole. For narcotization I used a 0.06 per cent solution of chloretone in tap water and the melanophores reacted as given in the following experiment.

Several tadpoles were examined under the binocular dissecting microscope and the condition of the melanophores noted. The animals were then placed in a 0.06 per cent solution of chloretone in tap water and left there for 3 hours, the longest time during which the experimental animals would be subjected to the chloretone solution. On examination it was found that the state of the melanophores was unchanged.

The discrepancy between Cole (1922) and Kropp (1927) and the present investigations hinges undoubtedly on the difference in chloretone concentration used, the weaker (0.06 per cent) solution evidently having no effect on the pigment cells. This is, of course, significant in light of the experiments to be cited.

Although many investigators have reported that the tadpole eyes are the mechanism for adaptive color changes, the behavior of the melanophores in blinded animals varies according to species (Kropp, 1927, 1929). For this reason before any experimental work on grafts was begun the color responses of blinded animals in the two species used for experimentation was studied.

The procedure was as follows: The tadpoles were anesthetized by

immersion in approximately 0.06 per cent chloretone for 15 minutes. A slit was then made in the cornea, the eyeball carefully lifted out, and then the muscles and optic nerve cut. In no case was the eyeball itself ruptured or injured. After the operation the animals were placed in tap water either in a white or in a black dish, where they were kept 12–16 weeks, with controls whose pigmentation was about like that of the experimental animal. In some cases the animal, along with a control, was placed alternately in a light and a dark background and the activities of the melanophores noted over a period of several weeks. The results, which refer only to the tail melanophores, may be summarized as follows: When young tadpoles were placed in a white dish immediately after the operation, the melanophores expanded slightly during the first 24 hours. For about 4–5 days the pigment cells remained in this slightly expanded state, then followed a gradual expansion to the reticulate state. This required about 2 weeks. This fully expanded state continued for as long as 10 weeks, at which time the observations were discontinued. If the animal was placed in a dark background in diffused light, the melanophores became stellate within 24 hours and the subsequent behavior was similar to that of tadpoles in a white background. In about 2 weeks the color cells were permanently reticulate. With old tadpoles immediate exposure of blinded animals to a white background brought about a punctate state which lasted about 10 days, followed by a gradual expansion over a period of 1 month to the stellate state. This persisted for over 8 weeks or until observations were stopped. In one case the animal assumed a semi-reticulate state after 4 months, indicating that probably the melanophores of all blinded tadpoles become eventually permanently expanded. On immediate removal to a black environment the color cells become stellate within 24 hours, remaining so for as long as the experiment lasted (8 weeks).

Another group of blind tadpoles (old) was placed alternately in the light and dark to see if the melanophores changed in amount of expansion when subjected to changes in environment. The results showed only a slight change (Fig. 1, *a* and *b*). The pigment cells contracted slightly in a white background. This lasted approximately 10 days. After this they showed little or no tendency to contract in

the light. These results indicate that the eyes in this species are the chief organs for color changes, although there is a slight reflex effect from the light receptors in the skin on the melanophores.

After these data were obtained the next series of experiments consisted in a study of the activities of the melanophores in transplanted skin. The method, essentially that recommended by Cole (1922) and Rand and Pierce (1932), was this: The animal was narcotized by immersion in approximately a 0.06 per cent solution of choretone in tap water. When activity ceased the animal was transferred to 0.01 per cent chloretone, in which solution the operation was performed. All operating was done with the aid of a binocular dissecting microscope. A square of skin approximately 4×6 mm. in size (depending somewhat on the size of the tadpole) was lifted from the tail, floated on a clean coverslip, thus insuring a complete disconnection with the nervous system, and then replaced in its original position. At the conclusion of the operation the tadpole was immersed in 0.01 per cent chloretone with the patch area lifted out of the liquid and kept from drying by covering the animal with a piece of filter paper the edges of which extended into the chloretone solution. The tadpoles were kept under these conditions for from 1 to 3 hours, during which time the patch attached itself to the underlined tissues. At this time the animal was lifted out of the chloretone solution and carefully immersed in an appropriately colored dish contain-

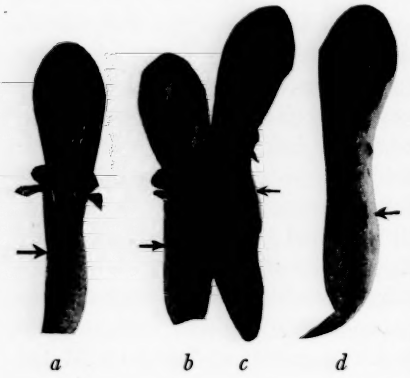

FIG. 1.—Homoiotransplant; arrows indicate position of patch. Animal Nos. 23*a* and 23*b*: *a* and *b*—animal No. 23*a*—blinded; *c* and *d*—animal No. 23*b*—normal. *a* and *d*, photographed together, animals light adapted; *b* and *c*, photographed together, animals dark adapted. The photographs show that the homoiotransplants undergo the same color changes as the host skin. Note also that *a* and *b* have not changed color to any appreciable extent although they were in white and black backgrounds, respectively, while *c* and *d* under the same conditions showed a very marked color change.

ing tap water, and the whole was removed to a source of diffused light.

An observation which May (1924) noted for reptilian skin was also seen in my amphibian autotransplants. Thus a graft in which a vascular supply is not established within a few hours soon becomes wrinkled, dies, and falls off—or, to be brief, does not "take." This immediate flooding with blood of the area below in a successful transplant is important in light of the behavior of the melanophores as cited below.

Twenty successful operations were performed and the activities of the melanophores in the patches studied and compared with those of the rest of the tail skin when the tadpole was subjected to light and dark backgrounds. In every case the patch melanophores responded like the rest of the tail pigment cells. Thus if immediately after operation the animal was placed in a white dish all the melanophores—i.e., those on the patch and those on the parent-skin—were punctate after 24–36 hours, no lag being observable in the transplanted area (Fig. 2). When this punctate individual was placed in a black dish in diffused light, all the melanophores became completely expanded after from 24 to 36 hours and remained so as long as the individual was kept in the black dish. Transfer to a white dish again brought about a punctate condition, and this change could be made an indefinite number of times with the usual changes in melanophore condition. Since it is highly improbable that nerves could grow into the transplanted patch within 24 hours, one may conclude that the melanophore changes in the transplanted area have been brought about by hormones in the blood stream or body fluids whose production is initiated through the activities of the eye (Kropp, 1929). This is contrary to the results noted by May (1924) for the activities in transplanted reptilian skin (*Anolis carolinensis* Cuv.). His experiments showed conclusively that a vascular supply alone failed to

FIG. 2.—Autotransplant; arrows indicate position of patch. Animal No. 22: *a*, light adapted; *b*, dark adapted.

bring back the color-changing reaction and that "the gradual regaining of full function by the melanophores . . . . can be correlated only with the slow regeneration of nerves."

One other series of experiments was made. In 1929 Kropp attempted by blood-transfusion methods to test the hypothesis that in normal color changes the blood carried a melanophore-activating material. With this idea in mind, the following experiment was devised to test this hypothesis: Two tadpoles of about the same coloring and same size were used. One individual was blinded by carefully removing the eyes. After the operation the animals were placed in a white dish for 10 days to permit the operation shock to wear off. At the end of this time examination under the binocular microscope showed that the melanophores of the normal animal were in a punctate condition while those of the blinded animal were slightly expanded. The two individuals were then chloretoned, patches removed from the tails of the two tadpoles, and the patches interchanged, so that we obtained the following: The blinded semi-stellate animal had on its tail a punctate patch obtained from the normal animal; the normal punctate tadpole had on its tail a semi-stellate patch. The first indications were that these homoiotransplants had "taken." Thus within a few hours after the operation the "foreign" patch showed a high degree of vascularity—blood from the host flooded the area beneath. This "taking," however (as will be shown), was only temporary, but it was of sufficient duration (approximately 2 weeks) to permit the study of the activity of live foreign melanophores flooded by blood from the host tadpole. After about 2 weeks the homoiotransplant showed signs of undergoing changes. What these changes were is uncertain; but examination of the living animal showed a peculiar gray transparency of the patch, a seeming degeneration of the melanophores, and the edges of the patch appeared curled up away from the host skin. The gradual replacement (Rand and Pierce, 1932) of the graft by host skin was rather slow, some of the graft skin persisting for at least 3 months.

Twenty-four hours after the homoiotransplantation the two individuals were placed in a white dish in diffused light. The next day the tail melanophores of the normal animal were punctate, even those which were on the foreign patch, while the melanophores of

the blinded animal were semi-stellate (Fig. 1, *a* and *d*). If these animals were now placed in a black dish under a diffused light, the blinded animal showed no change while the normal animal became reticulate over its entire surface (Fig. 1, *a*, *b*, and *c*). Thus we have a method for flooding isolated patches of skin with the hormone directly after it is produced by the donor tadpole. The foreign patch responds as the host skin for approximately 2 weeks. At that time the transplanted melanophores assume an expanded condition which persists until the patch is eventually absorbed and replaced (Rand and Pierce, 1932).

Another series of experiments of the same nature, except that neither animal was blinded, gave similar results. The foreign skin behaved as the host skin for a period lasting approximately 2 weeks. After that time degenerative or other changes produced a gradual breakdown of the melanophore cells, with associated non-response to the color changes in environment.

These homoiotransplants seem, in my opinion, to prove very definitely the humoral nature of melanophore control in tadpoles. It is highly improbable that nerves from the host would grow into the foreign patch, but it is easy to see that blood, lymph, etc., from the donor tadpole with its melanophore controlling hormones might activate the pigment cells of the foreign skin, at least for as long as no degenerative or other changes take place there.

SUMMARY

1. Expansion or contraction of the melanophores in *Rana areolata* requires approximately 36 hours for completion.

2. Exposure of the tadpoles to a 0.06 per cent chloretone solution in tap water for 3 hours has no effect on the melanophores.

3. The melanophores of blinded tadpoles behave as follows: (*a*) in young tadpoles the melanophores after approximately 2–3 weeks assumed a permanently reticulate state regardless of the color of the environment; (*b*) in old tadpoles the melanophores after 4–5 weeks assumed a permanently (?) stellate condition regardless of the color of the environment.

4. In autotransplants, before nerve elements grow into the graft, the melanophores show color changes like the rest of the skin.

5. In homoiotransplants the melanophores react like the host tadpoles for approximately 2 weeks, then follow degenerative or other changes with associated non-response to the color changes in the environment.

6. These results indicate that the melanophores in *Rana areolata* can be activated by substances carried in the blood stream.

LITERATURE CITED

COLE, W. H. 1922. The transplantation of skin in frog tadpoles, with special reference to the adjustment of grafts over eyes, and to the local specificity of integument. Jour. Exp. Zoöl., **35**:353–419.

HOGBEN, L. T. 1924. The pigmentary effector system. Edinburgh.

KROPP, B. 1927. The control of the melanophores in the frog. Jour. Exp. Zoöl., **49**:289–318.

———. 1929. The melanophore activator of the eye. Proc. Nat. Acad. Sci., **15**:693–94.

MAY, R. M. 1924. Skin grafts in the lizard, *Anolis carolinensis* Curo. Brit. Jour. Exp. Biol., **1**:539–55.

PARKER, G. H. 1930. Chromatophores. Biol. Rev., **5**:59–90.

———. 1932. Humoral agents in nervous activity. Cambridge.

PERKINS, E. B., and B. KROPP. 1932. The crustacean eye hormone as a vertebrate melanophore activator. Biol. Bull., **63**:108–12.

RAND, H. W., and PIERCE, M. E. 1932. Skin grafting in frog tadpoles: Local specificity of skin and behavior of epidermis. Jour. Exp. Zoöl., **62**:125–70.

ADDITIONAL OBSERVATIONS ON HETEROPLASTIC CHORIO-ALLANTOIC GRAFTS OF EMBRYONIC DUCK KIDNEY TISSUE

(Four figures)

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THE problem of heteroplastic transplantation in warm-blooded vertebrates has attracted much attention, but little advance has actually been made in determining the factors responsible for the failure of the tissues of one species to grow when transplanted to another. Usually, the transplantations have been made between adults. Since normal growth of tissue is possible in heteroplastic transplants when both the host and donor are embryonic, grafting to the chorio-allantoic membrane of the avian embryo seems to offer a more promising method of attack. It enables the study of both the donor tissue and the host during the period when either, or both, are developing those properties which make their tissues incompatible.

Murphy (1914–26) was the first to recognize chorio-allantoic grafting as a valuable technique in this respect. His grafts of embryonic and sarcomatous tissue of the rat on the chick membrane gave evidence that the host became resistant toward foreign tissue immediately after the eighteenth day of incubation. He found that the chorio-allantoic membrane was an excellent place to grow tissue before this time, regardless of either the age or species of the donor.

Sandstrom (1932*a*, 1932*b*) obtained results from the transplantation of duck kidney tissue to the chorio-allantoic membrane of the chick which failed to support some of the conclusions of Murphy. The capacity of the duck tissue to grow on the chick membrane was found to be correlated with the degree of differentiation of the donor tissue at the time of transplantation. It is true that all of the grafts were removed from the host on the seventeenth day of incubation, i.e., before the development of a resistance in the host as determined by Murphy; nevertheless, the differentiated kidney tissue became

necrotic and infiltrated with connective tissue. Since the results of homoplastic transplantations of duck kidney tissue to the chorio-allantoic membrane of the duck were practically the same, no definite conclusion concerning the rôle of species specificity as a factor in the results of heteroplastic transplantations was possible. Function was suggested as an important factor in the success of either type of graft, and recently Sandstrom and G. L. Kauer (1933*a*), and Sandstrom and J. T. Kauer (1933*b*), have obtained results from the transplantation of cartilage and macerated duck kidney tissue, respectively, that corroborate the earlier findings and suggestions of Sandstrom.

The results of the previous transplantations of duck kidney tissue to the chick membrane were so encouraging, and suggested so many possibilities, that further experiments were planned. The transplants considered in this paper (reported in abstract; Sandstrom, 1932*c*) are the result of experiments designed to discover, if possible, something of the nature of the host-donor reactions in heteroplastic transplantations during embryonic development.

MATERIAL AND METHODS

The method of transplanting the donor tissue to the chorio-allantoic membrane of the host was done in practically the same manner as that described by Willier (1924), who has improved the technique of chorio-allantoic grafting considerably since it was first used by Rous and Murphy (1912). A few changes were necessary to suit the particular needs of the present experiments; but, in general, the procedure was very much like that described by Sandstrom (1932*a*).

The hosts for the heteroplastic transplants were single-comb White Leghorn chick embryos which had been incubated 9 days. The donors were White Pekin duck embryos whose ages were 9, 13, 19, and 24 days of incubation. It was deemed inadvisable to use older donors, as earlier work (Sandstrom, 1932*a*, 1932*b*) indicated that they would not add materially to the results obtained from the 24-day transplantations. Metanephric tissue was used in all cases.

Homoplastic transplantations were also made for comparison to discover any indications that species specificity might be responsible for the results obtained in the heteroplastic grafts. They consisted of

two representative series, the donors of which were of 13 and 21 days' incubation, and the hosts 14-day duck embryos.

In both types of transplantations the grafts were removed at 24-hour intervals following an initial period of 48 hours, which permitted adequate incorporation of the implant into the host membrane. The grafts were fixed in Zenker's acetic solution, sectioned serially at 8 μ, and stained in methylene blue-eosin.

RESULTS

A. GRAFTS OF DUCK KIDNEY TISSUE ON THE CHORIO-ALLANTOIC MEMBRANE OF THE CHICK

1. *Donors of 9 days' incubation.*—The metanephros of the 9-day donor is a loosely bound organ consisting of considerable undifferentiated metanephrogenous tissue in which a few collecting and secreting tubules are in the process of formation. At this stage of development, the metanephric tissue grew and differentiated normally when implanted on the chorio-allantoic membrane of the chick. This series consisted of twenty-seven grafts which were removed at intervals from the second to the ninth day, when the experiment was terminated. In all cases, with one exception, the tissue was not visibly affected by the new environment; it appeared histologically normal. The exception, removed from the host on the eighth day, showed a very small area of necrotic tissue in the center of the graft. The grafts, which were removed after remaining only 2 days on the host membrane, gave evidence of a slight inhibition of differentiation. This inhibition, however, was probably due to conditions present during the process of incorporation of the implant into the host, and was not noticeable in the grafts removed at later intervals. In the latter grafts there was a marked advance in differentiation to a degree which corresponded very closely to that of the kidney in its normal development. Numerous collecting and secreting tubules, and many renal corpuscles, were present, while considerable differentiating metanephrogenous tissue still remained about the periphery.

The hosts, which varied in age from 11 to 18 days of incubation at the time the grafts were removed, had developed no antagonism toward the foreign tissue. Connective tissue entered the grafts in the

early stages of incorporation to fill the spaces between the areas of differentiating tissue; but neither infiltration nor encapsulation, such as is usually described in foreign tissue grafts, was found. Lymphocytes appeared in increased numbers only in one case, the graft of which was removed on the seventh day; and a few granulocytes were found at irregular intervals throughout the series, mainly among the older tissues.

2. *Donors of 13 days' incubation.*—On the thirteenth day of incubation the metanephros of the duck is a somewhat compact structure. Many tubules, both secreting and collecting, and renal corpuscles are formed; and differentiating metanephrogenous tissue is still present about the periphery. The relative amounts of differentiated and undifferentiated tissue in the pieces implanted to the chorio-allantoic membrane of the chick depended on the part of the metanephros from which the piece came, and varied in the different implants, thus accounting for the variations in the results of any one group of grafts.

In general, the results of the transplantations of the 13-day donor tissue, as listed in Table I, show considerable differences when compared with those obtained from 9-day donors. Although most of the tissue was normal in the grafts removed on the second and third days, necrosis was present in several. It was limited, however, to the central part of the transplant and usually consisted of pink-staining tubules, whose structure was abnormal. The tissue about the periphery appeared normal. In the grafts removed on the fourth day, the infiltration of the lymphocytes was so intense that it was impossible to determine definitely the status of the metanephric tissue (Fig. 1). Much of it seemed normal, especially that around the periphery where the tubules were visible; but the centrally located areas stained pink, making a contrasting background for the numerous lymphocytes.

As indicated in Table I, normal tissue was found in the grafts removed subsequent to the fourth day, with possible exceptions in those removed the last two days, i.e., the tenth and eleventh days, which will be discussed more fully presently. The tissue appeared, for the most part, like that of the normally developing organ of embryos of equivalent ages (Fig. 2). In some of the grafts removed on

the eighth, ninth, and tenth days distended tubules were present, indicating that the metanephric tissue, equivalent in age to that of normal embryos of 21, 22, and 23 days' incubation, respectively, was

TABLE I

GRAFTS OF 13-DAY DUCK KIDNEY TISSUE MADE TO THE CHORIO-ALLANTOIC MEMBRANE OF THE 9-DAY CHICK
(SERIES 201)

GRAFT NOS.	LENGTH OF TIME ON HOST (DAYS)	AGE OF HOST AT TIME OF REMOVAL (DAYS)	AGE OF TISSUE IN GRAFT AT TIME OF REMOVAL (DAYS)	CONDITION OF TISSUE IN GRAFT	CELLULAR REACTION		
					Connective Tissue	Lymphocytes	Granulocytes
1	2	11	15	Apparently all normal	−	+	+
2–4	2	11	15	Mostly normal; few scattered necrotic areas	−	+	−
5	2	11	15	Mostly necrotic	−	+	−
6	3	12	16	Mostly normal	+	−	+
7	3	12	16	Apparently all normal	+	+	+
8	3	12	16	Considerable normal	−	+	+
9–11	3	12	16	Completely normal	−	−	+
12	4	13	17	Mostly normal	−	+	+
13–15	4	13	17	Mostly necrotic	−	+	+
16–21	5	14	18	Completely normal	−	−	+
22–23	6	15	19	Completely normal	+	−	+
24	6	15	19	Completely normal	−	−	+
25–27	7	16	20	Completely normal	−	−	+
28–31	8	17	21	Completely normal	−	−	+
32–34	9	18	22	Completely normal	−	−	+
35	10	19	23	Apparently normal tubules in a large blood clot	−	−	?
36	10	19	23	Completely normal	−	−	+
37	10	19	23	Apparently normal tubules in a large blood clot	−	+	−
38	11	20	24	Same as preceding	−	+	−
39	11	20	24	Doubtful tissue in a large blood clot	?	?	?

functional. Although not yet determined, it is quite probable that portions of the normal organ are active at this time.

The appearance of numerous small lymphocytes in many of the grafts removed from the chick membrane on the second, third, and fourth days, but infrequently thereafter, is of considerable interest. The hosts bearing these grafts ranged in age from 11 to 13 days. As

PLATE I

FIG. 1.—Case 201-13. Photomicrograph to show intense lymphocytic infiltration in, and about, a graft of 13-day duck kidney tissue removed after having grown for 4 days on the chorio-allantoic membrane of the chick. Host of 13 days' incubation at the time of removal. ×80.

FIG. 2.—Case 201–30. Photomicrograph to show normal differentiation of 13-day duck kidney tissue after having grown for 8 days on the chick membrane. Host of 17 days' incubation at the time of removal. ×40.

PLATE II

FIG. 3.—Case 201–37. Photomicrograph to show extravasated blood in graft of 13-day duck kidney tissue after having grown for 11 days on the chick membrane. Host of 20 days' incubation at the time of removal. ×40.

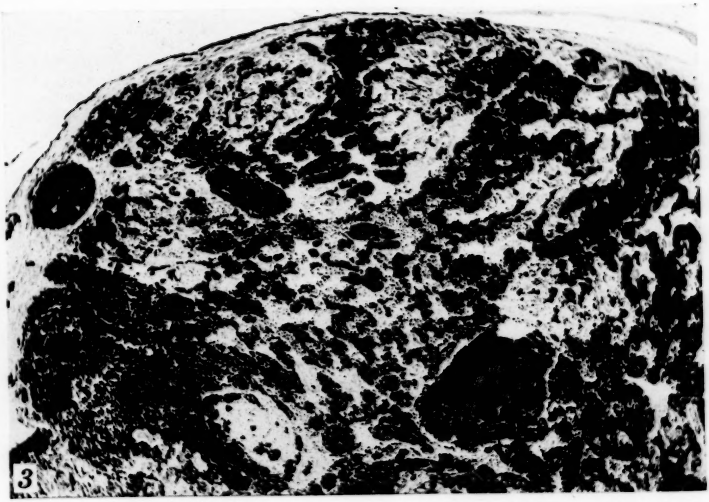
FIG. 4.—Case 85–49. Photomicrograph to show normal, functional metanephric tissue equivalent in age to that of a 2-day-old duckling which differentiated on the chorio-allantoic membrane of the chick. Age of the donor at the time of transplantation was 19 days of incubation. The graft was allowed to grow on the host 11 days. Age of the host at time of removal was 20 days of incubation. Photomicrograph shows only a small part of the graft which consists of completely normal tissue and a large vesicle (*b*), only a part of which is shown. Note the large collecting ducts (*a*) which are continuous in the graft and connect the uriniferous tubules with the large reservoir-like vesicle. No signs of a host reaction appear in the host tissue (*c*). ×40.

PLATE I

1

2

PLATE II



previously mentioned, the lymphocytes were often so abundant that it was almost impossible to see the metanephric tissue in some parts of the graft (Fig. 1). Their greatest intensity was in the central portions where the transplanted tissue appeared necrotic. None were found in the grafts removed on subsequent days until the tenth and eleventh days, but in these later grafts they were never as numerous as in the earlier.

Unlike the lymphocytes, the granulocytes appeared in many of the grafts regardless of the time they were removed. There was no apparent correlation between their numbers and the length of time the transplants remained on the host. They were never present in large numbers.

It seems advisable to make comments here with respect to certain irregularities obtained in the grafts that remained on the chick membranes for 10 days or longer, the host embryos of which were 19 days or older. Normally the chick hatches on the twenty-first day of incubation, but variations occur, so that hatching is often either on the twentieth or twenty-second day. Since the chorio-allantoic membrane is the embryonic respiratory organ, to be discarded shortly before hatching, certain changes sometimes occur in it as early as the nineteenth day which make it unsuitable for the growth of transplanted tissue. Therefore, the grafts removed during this period may have been growing under unsuitable conditions. An example of such is Graft 37, which was removed from a host 19 days of age, after it had grown for a period of 10 days (Fig. 3). The blood vessels of the chorio-allantoic membrane immediately surrounding the graft were densely packed with red blood corpuscles, as were also the blood vessels within the graft. In addition, the blood cells were thickly packed in the spaces between the metanephric tubules of the transplanted tissue, which in many instances still appeared in a normal condition. Occasionally lymphocytes appeared. The extreme reaction was obtained in those grafts removed on the twentieth day, where the tissue was located in a large blood clot. On the other hand, there were cases (Graft 36, for example) in which both the membrane and the graft were normal in every respect.

3. *Donors of 19 days' incubation.*—The metanephros of the 19-day duck embryo shows considerable growth and a marked decrease in

the relative amount of undifferentiated metanephrogenous tissue. The pieces transplanted, therefore, consisted mostly of differentiated tubules, although varying amounts of undifferentiated tissue were also included. As mentioned in connection with the previous series, this heterogeneous condition of the implant has a distinct bearing on the results, and accounts for the variation in the different groups.

The general results which are included in Table II show striking differences when compared with those of the last series. There was an increase both in the percentage of grafts containing necrotic tissue and in the amount of necrosis found in the individual grafts. In fact, necrotic tissue was found in grafts distributed throughout the entire series, although normal tissue was present in most of them, and constituted almost entirely the grafts removed on the ninth, tenth, and eleventh days. Of these, Grafts 45 and 49 are of considerable interest. The latter graft was removed from the membrane of a 20-day embryo, at which time the grafted tissue was equivalent in age to the metanephros of a 2-day old duckling. The large graft was made up of normal metanephric tissue (Fig. 4) and an extensive vesicle which was probably a very much distended tubule. Leading into the vesicle were a number of large collecting tubules which received numerous smaller tubules from all parts of the graft. There is no doubt that the transplant consisted of a completely integrated functional unit, the secretions of which were collected in the large bladder-like vesicle. The same conditions were found in Case 84–9, except that in this instance the host was of 19 days' incubation, making the transplant equivalent to metanephric tissue from a duckling one day old. Other grafts showed similar conditions but were not as striking as the examples given.

Like the previous series, the early stages of incorporation disclosed small lymphocytes in great abundance, especially in the central necrotic areas of the grafts. However, in this series they persisted for a longer time and were found as late as the sixth day, though rarely thereafter. Granulocytes were present in varying numbers. Seldom did they appear in great numbers, and it was impossible to correlate their prevalence or distribution with the condition of the grafted tissue or the age of the host.

TABLE II

GRAFTS OF 19-DAY DUCK KIDNEY TISSUE MADE TO THE CHORIO-ALLANTOIC MEMBRANE OF THE 9-DAY CHICK EMBRYO
(SERIES 76–86)

GRAFT NOS.	LENGTH OF TIME ON HOST (DAYS)	AGE OF HOST AT TIME OF REMOVAL (DAYS)	AGE OF TISSUE IN GRAFT AT TIME OF REMOVAL (DAYS)	CONDITION OF TISSUE IN GRAFT	CELLULAR REACTION		
					Connective Tissue	Lymphocytes	Granulocytes
1–2	2	11	21	Mostly normal; scattered necrotic tubules	−	+	+
3–4	2	11	21	Considerable necrotic	−	+	+
5	2	11	21	Mostly necrotic	−	+	−
7–10	3	12	22	Mostly normal; scattered necrotic tubules	−	−	+
11	3	12	22	Mostly necrotic	−	+	+
12	3	12	22	Mostly normal	−	−	+
13	3	12	22	Mostly normal	+	−	+
14	3	12	22	Mostly normal	+	+	+
15	4	13	23	Mostly normal	+	−	+
16	4	13	23	Mostly normal	−	+	+
17	4	13	23	Considerable necrotic	+	+	+
18	5	14	24	Equal amounts of normal and necrotic	+	+	+
19	5	14	24	Mostly normal	+	−	+
20	6	15	25	Mostly necrotic	+	+	+
21	6	15	25	Mostly normal	−	−	+
22–23	6	15	25	Mostly necrotic	+	−	+
24–25	6	15	25	Mostly normal	+	−	+
26	6	15	25	Mostly necrotic	+	−	−
27	7	16	26	Mostly necrotic	+	−	+
28	7	16	26	Mostly normal	+	−	+
29	7	16	26	Completely normal	−	−	−
30–32	7	16	26	Completely normal	−	−	+
33	8	17	27	Completely normal	−	−	+
34	8	17	27	Mostly necrotic	+	−	+
35–36	8	17	27	Mostly normal	+	−	+
37	8	17	27	Completely necrotic	+	−	−
38	9	18	28	Equal amounts of necrotic and normal tissue	+	+	+
39–42	9	18	28	Apparently all normal	−	−	+
43	9	18	28	Mostly normal	+	−	+
44–47	10	19	29	Apparently all normal	−	−	+
48	10	19	29	Mostly normal; small scattered necrotic areas	−	−	+
49–54	11	20	30	Completely normal	−	−	+
55	12	21	31	Condition of tissue doubtful	+	+	+

TABLE II—*Continued*

Graft Nos.	Length of Time on Host (Days)	Age of Host at Time of Removal (Days)	Age of Tissue in Graft at Time of Removal (Days)	Condition of Tissue in Graft	Cellular Reaction		
					Connective Tissue	Lymphocytes	Granulocytes
56	12	21	31	Blood in spaces between tubules; condition of tissue doubtful	−	?	?
57	12	21	31	Same as preceding	−	+	+
58	12	21	31	Considerable normal	+	+	+
59	12	21	31	Both normal and necrotic present; blood in spaces between tubules	+	−	+
60	12	21	31	Mostly normal; blood appearing in spaces	−	−	+

The connective tissue reaction against the transplants was quite definite. Although it was found in grafts removed as early as the third day, it did not occur with any great intensity until after the sixth, and then only when necrotic tissue was present. Also, there seemed to be a correlation between the absence of lymphocytes and the presence of connective tissue. In most of the grafts the latter was found only when the infiltration of lymphocytes was slight or absent.

A number of grafts removed on the twelfth day, when the host was of 21 days' incubation, showed conditions similar to those described in the previous series, and are interpreted as the result of changes in the chorio-allantoic membrane just previous to hatching.

4. *Donors of 24 days' incubation.*—Because of the advanced state of differentiation in the metanephros of the 24-day duck embryo, the implants consisted for the most part of completely differentiated tissue. Relatively little undifferentiated metanephrogenous tissue remains.

The results, listed in Table III, can be described briefly, for their differences are in degree and not in kind, when compared with those obtained from the transplantation of tissue from 19-day donors. Without exception, every graft in the series contained some necrotic

TABLE III

GRAFTS OF 24-DAY DUCK METANEPHRIC TISSUE MADE TO THE CHORIO-ALLANTOIC MEMBRANE OF THE 9-DAY CHICK EMBRYO
(SERIES 88–99)

GRAFT NOS.	LENGTH OF TIME ON HOST (DAYS)	AGE OF HOST AT TIME OF REMOVAL (DAYS)	AGE OF TISSUE IN GRAFT AT TIME OF REMOVAL (DAYS)	CONDITION OF TISSUE IN GRAFT	CELLULAR REACTION		
					Connective Tissue	Lymphocytes	Granulocytes
1–2	2	11	26	Mostly necrotic	−	+	−
3	2	11	26	Scattered areas of normal	−	+	−
4–8	2	11	26	Scattered areas of normal	+	+	−
9–10	3	12	27	Scattered areas of normal	+	+	−
11	3	12	27	Mostly normal	−	−	+
12	3	12	27	Mostly necrotic	−	+	−
13	3	12	27	Considerable normal	+	+	−
14	4	13	28	Considerable normal	−	+	+
15	4	13	28	Considerable normal	+	−	+
16–17	4	13	28	Some normal; some necrotic	+	−	−
18–19	4	13	28	Mostly necrotic	+	+	−
20	6	15	30	Few normal tubules at periphery; mostly necrotic	+	−	+
21–22	6	15	30	Same as preceding	+	+	+
23	7	16	31	Completely necrotic	+	+	−
24	7	16	31	Completely necrotic	+	+	+
25	7	16	31	Completely necrotic	−	−	+
26	7	16	31	Few normal tubules; remainder necrotic	−	+	+
27	7	16	31	Completely necrotic	+	−	+
28	7	16	31	Few normal tubules; rest necrotic	+	−	−
29	7	16	31	Same as preceding	−	−	−
30	8	17	32	Small areas of normal; rest necrotic	−	+	+
31	8	17	32	Mostly necrotic	+	+	+
32	8	17	32	Few normal tubules; rest necrotic	+	−	−
33	8	17	32	Same as preceding	+	−	+
34	9	18	33	Some normal; some necrotic	+	−	+
35–37	9	18	33	Considerable normal	+	−	+
38	11	20	35	Few normal tubules; rest necrotic	+	−	−
40	11	20	35	Completely necrotic	+	+	−
41	11	20	35	Completely necrotic	+	−	−
39	11	20	35	Mostly necrotic	+	−	+

TABLE III—*Continued*

Graft Nos.	Length of Time on Host (Days)	Age of Host at Time of Removal (Days)	Age of Tissue in Graft at Time of Removal (Days)	Condition of Tissue in Graft	Cellular Reaction		
					Connective Tissue	Lymphocytes	Granulocytes
42	11	20	35	Some normal; some necrotic	+	−	+
43	12	21	36	Blood in spaces between tubules; condition of tissue doubtful	+	?	?
44	12	21	36	Some normal; some necrotic	+	+	+
45	12	21	36	Few necrotic tubules; remainder of graft connective tissue	+	−	−
46	12	21	36	Completely necrotic	+	+	+

tissue; and in general, the amount in the individual grafts was found to be considerably greater.

Small lymphocytes appeared in many of the grafts removed on, or before, the sixth day, and also to a lesser degree in those removed at later intervals. They were most dense in the transplants that were removed on the second, third, and fourth days, and not uncommonly appeared in lesser numbers, usually in localized areas of necrosis in other instances. Granulocytes were found in grafts removed at all stages and were rarely abundant. In general, they seemed to be less numerous than in the grafts removed from 19-day donors.

The connective tissue reaction toward the implanted tissue was similar to that described for the previous series. It manifested itself with greatest intensity in those grafts which showed no lymphocytes.

B. GRAFTS OF DUCK KIDNEY TISSUE ON THE CHORIO-ALLANTOIC MEMBRANE OF THE DUCK

1. *Donors of 13 days' incubation.*—The results of the transplantation of 13-day donor tissue to the chorio-allantoic membrane of the duck are, in general, much like those of the same-aged donors implanted on the chick membrane. There were twenty-eight grafts in this group. All of the tissue was normal except in the grafts removed

from the host during the early stages of incorporation. In these, varying amounts of apparently necrotic tissue were present. No necrosis was found in the grafts removed in the later stages.

The reactions of the host toward the transplanted tissue were not, as a whole, very pronounced. A lymphocytic infiltration was present in the transplants removed on the second day, but nothing resembling an infiltration was present in any of the others. A connective tissue invasion was found in but a single instance; and granulocytes, although found distributed throughout the entire series, showed no consistent increase or decrease in number with the increased length of time the donor tissue remained on the host.

2. *Donors of 21 days' incubation.*—The grafts obtained in this group of transplantations resembled, in their histological structure, those obtained from the transplantation of 19- and 24-day donor tissue made to the chick membrane. The results are tabulated in Table IV. The amounts of normal and necrotic tissue varied throughout the series, owing, as previously mentioned, to the simultaneous presence of undifferentiated and differentiated metanephric tissue during the normal development of the organ.

The reaction of the host differed from that in the heteroplastic transplants (compare Table IV with Tables II and III) only in the fact that the lymphocytic infiltrations were not found after the third day with but one exception, Graft 10; and in this, the incorporation of the implant was incomplete and the vascularization poor. Usually, connective tissue was present only in grafts having necrotic tissue. The relation between the connective tissue and the small lymphocytes was similar to that described for the heteroplastic grafts. Granulocytes, as in all other grafts described thus far, were common, but showed no consistent relationship with other observable facts.

DISCUSSION

That the capacity for growth of duck metanephric tissue in grafts on the chorio-allantoic membrane of either the chick or duck is dependent on the degree of differentiation of the donor tissue at the time of transplantation, as contended by Sandstrom (1932*a*), is fully verified by the results of the present experiments. This conclusion is demonstrated by the relative amounts of normal and

necrotic tissue obtained in the grafts. Transplants made from the metanephros of 9-day duck embryos yielded grafts that were normal at all stages of removal, while tissue obtained from older donors yielded grafts with increasing amounts of necrosis.

TABLE IV

GRAFTS OF 21-DAY DUCK METANEPHRIC TISSUE MADE TO THE CHORIO-ALLANTOIC MEMBRANE OF THE 14-DAY DUCK (SERIES 205)

Graft Nos.	Length of Time on Host (Days)	Age of Host at Time of Removal (Days)	Age of Tissue in Graft at Time of Removal (Days)	Condition of Tissue in Graft	Cellular Reaction		
					Connective Tissue	Lymphocytes	Granulocytes
1–2	2	16	23	Apparently mostly necrotic	+	+	−
3	2	16	23	Apparently mostly necrotic	−	+	−
4–5	3	17	24	Mostly normal; scattered necrotic areas	+	+	+
6	3	17	24	Same as preceding	−	−	+
7–9	4	18	25	Almost completely normal	+	−	−
10	5	19	26	Considerable necrotic	+	+	−
11	5	19	26	Almost completely normal	+	−	−
12	6	20	27	Completely normal	−	−	−
13	6	20	27	Almost completely normal	+	−	−
14	7	21	28	Completely normal	+	−	+
15	7	21	28	Mostly normal; small necrotic areas	+	−	+
16	8	22	29	Some normal; some necrotic	+	−	−
17	8	22	29	Mostly normal; small necrotic areas	+	−	+
18	8	22	29	Completely normal	−	−	+
19	9	23	30	Completely normal	−	−	+
20–21	9	23	30	Mostly necrotic	+	−	−
22	11	25	32	Mostly normal	+	−	+

An analysis of the heteroplastic, and also the homoplastic, grafts permits at least two explanations for the appearance of necrosis: first, the rate at which the process of incorporation[1] takes place; and

[1] "Process of incorporation" is used in this paper to denote, not only the surrounding of the implant by the connective tissue of the host, but also the vascularization of the grafted tissue by the blood vessels of the host.

second, the inability of the grafted tissue to function in its new environment. That the process of incorporation has an effect on the grafted tissue is substantiated by the study of the grafts from the variously aged donors removed during the early stages. The metanephric tissue from the 9-day donor is loosely bound. Within 2 days it became fully incorporated into the membrane, i.e., the loose connective tissue of the host had passed into the spaces of the donor tissue so that complete vascularization was possible within a very short time. The tissue was capable of continued growth with very little delay. This was not true for the implants of the older tissue. As the metanephros differentiates, it becomes a more and more compact structure, so that when portions of it are grafted, vascularization is not affected equally in all parts. Accordingly, the transplants which were removed during the early stages of incorporation (e.g., on the third and fourth days) had normal tissue about the periphery; whereas the more central regions contained tissue which was apparently necrotic because of insufficient vascularization. The results of Sandstrom and Kauer (1933*b*) are of interest in this respect. Implants of macerated duck kidney tissue from donors of 13, 21, and 27 days' incubation showed no centrally located areas of necrosis. Regardless of the age of the donor, the implant was made comparable to that of the 9-day donor mentioned above, since the compactness of the tissue was destroyed by the maceration, and rapid incorporation was permitted. Loeb (1920, 1926*a*, 1926*b*) noted that in grafts of kidney and thyroid made chiefly between mammalian adults, the center, being ill-nourished during the first few days, died, whereas the peripheral gland tissue survived. Minoura (1921) and Willier (1924) described the condition of gonad and thyroid tissue, respectively, in chorio-allantoic grafts, and noted that it was dependent on the rapidity and extent of the vascularization, particularly in the early stages of incorporation. More recently, Waterman (1932) transplanted embryonic rabbit primordia to the omental bursae of adults, and found that normal tissue was present when vascularization was immediate and kept abreast with the growth and differentiation of the graft. He also mentioned size of the transplant as a factor in preventing immediate vascularization during the early stages of incorporation.

The ultimate fate of the necrotic areas that were due to conditions present during the early stages of incorporation can only be conjectured from the sectioned material. In grafts obtained from 13-day donor tissue, the necrotic areas had disappeared by the fifth day. Without a doubt, absorption and phagocytosis were responsible for the disappearance of some of it, particularly that part formed from the differentiated tubules that were present at the time of implantation. The areas previously occupied by the necrotic tissue became filled, either with connective tissue which, if the areas were large enough, could easily be detected in the grafts removed during later stages, or by actively growing metanephric tissue, and therefore indiscernible at any time. However, it is possible also that the tissue in the early stages of incorporation was not actually necrotic, even though it had lost its identity through changes in structure and staining reaction, but was merely disorganized and still capable of sufficient recovery to continue normal development. Dedifferentiation and subsequent regeneration might be suggested, but evidence that either had occurred was not found. Saltykow (1900) was probably referring to this same general phenomenon when he stated that degeneration and regeneration took place in grafts of embryonic mammalian tissue made to adults of the same and different species.

The necrosis present in the grafts removed in the later stages was due in part to the persistence of some of the necrotic tissue mentioned above and in part to the inability of the kidney tissue to function properly after it had become differentiated on the host membrane. When function could be demonstrated, the tissue was always completely normal. The evidence of function in the grafts, to be sure, is not direct; and experiments to show active secretary function in chorio-allantoic grafts by means of vital dyes have not been entirely successful thus far. Nevertheless, results such as those obtained in Grafts 45 and 49 (Series 76–86) in which there are direct connections, by means of large collecting ducts, between uriniferous tubules and large vesicles formed from distended ducts, afford convincing evidence that the grafts are complete, functional units. The tissue of these grafts was normal, as it was also in numerous other grafts having less pronounced, but no less important, distended tubules.

That function is an important factor in determining the continued

growth of a tissue once it is fully incorporated by the chorio-allantoic membrane of the host was suggested by Sandstrom (1932*a*) in discussing the failure of duck kidney tissue to grow in chorio-allantoic grafts on either the chick or duck. More recently, Sandstrom and Kauer (1933*b*) obtained further confirmatory information from grafts of macerated kidney tissue of the duck on the chick membrane. Inability of the disorganized tissue to function efficiently brought on necrosis earlier, and more sporadically, than it occurred in grafts of intact tissue. Ruth H. Sandstrom (1934) suggested that the incapacity of liver and pancreas to continue growth in chorio-allantoic grafts was due to the inability of the tissue to function. She found that chicken liver tissue became necrotic at a time in development when certain changes take place in the embryo which might be related to the function of the liver. Furthermore, by means of specific histological stains, she observed that pancreatic tissue was never able to develop to a functional stage. Hunt (1932) observed that muscle tissue in homoplastic chorio-allantoic grafts of chick limbs showed a pronounced fatty degeneration which was more noticeable in the non-innervated grafts. Since no host reaction was found in the grafts, the lack of proper functional activity was probably responsible for the degeneration. On the other hand, Sandstrom and Kauer (1933*a*) showed that tissues, such as cartilage and bone, which have a passive function in the organism in contrast to the active function of kidney, liver, etc., would continue to grow. Retransplants of cartilage from variously aged duck embryos were made on the chick chorio-allantois, so that tissue equivalent to a 20-day duckling was removed after it had grown on the host for 27 days. Normal bone and cartilage made up the grafts, and surprisingly little necrosis was found.

The reaction of the host toward the transplanted tissue is of considerable interest. It is local and is confined to the tissues of the host immediately surrounding the transplant. With the exception of grafts obtained from 9-day donor tissue which had no lymphocytic infiltrations at any stage of removal, intense infiltrations of small lymphocytes appeared in both homoplastic and heteroplastic transplants. In the homoplastic series, they were present in the grafts of 13-day donor tissue up to, and including, the second day on the

host; and in the grafts of 21-day donor tissue, up to, and including, the fourth day. Seldom were they found thereafter. In contrast, they were found in the heteroplastic series of 13-day donor grafts as late as the fourth day, and in the 19-day grafts as late as the sixth day, but in neither were they observed commonly in the grafts that were removed at later intervals, except in some taken off on the tenth, eleventh, and twelfth days. In the grafts of 24-day donor tissue, intense lymphocytic infiltrations were present up to, and including, the sixth day, and with less intensity in a number of grafts thereafter.

There is little but circumstantial evidence to account for the presence of the numerous lymphocytes in the grafts, but their occurrence seems coincident with the period of incorporation when vascularization is poor. Supporting this contention is the complete absence of lymphocytes during the early stages of incorporation of metanephric tissue from 9-day donors where there is a rapid establishment of vascularization, and their presence in considerable numbers in the more centrally located regions of the grafts of the more compact tissue from older donors. Sandstrom and Kauer (1933*b*) found no lymphocytes in the grafts of macerated duck kidney tissue. This was probably due to the rapid vascularization which has already been noted. It might also be suggested that differences in intensity of infiltrations, and even their complete absence in some of the grafts, was due to the size of the piece transplanted; the smaller the piece, the more rapidly would it be incorporated. The presence of many lymphocytes in the grafts removed just prior to hatching can be explained on the same basis, for the circulation of the chorio-allantoic membrane, according to the evidence presented, is affected during the events incident to hatching. The density of the red blood cells in the chorio-allantoic vessels, and the great amount of extravasated blood within the grafts, indicates the condition of the circulation at this time. The presence of lymphocytes in the grafts of rat tissue obtained by Murphy (1914*a*, 1914*b*) might also be explained in this way. Loeb (1921), reviewing work which he and his co-workers had done over a period of years on the growth of tissue in transplants and the reactions against the transplanted tissue, postulated the formation of toxins (metabolic products) by the transplanted tissue. These toxins

diffused into the neighboring tissues of the host, stimulated the accumulation of lymphocytes, activated the connective tissue, and decreased the vascularization of the grafts. No effect on vascularization was noticed in the grafts of the present experiments, once they were fully incorporated; but the presence of the lymphocytes in the grafts that he obtained might possibly be explained in this manner.

In addition to the small lymphocytes, granulocytes and connective tissue appeared in response to the presence of the grafted tissue, whether homoplastic or heteroplastic. The granulocytes showed no consistent relationship between the age of the tissue transplanted, the time it remained on the host, or the age of the host. In this respect, the surrounding host tissue reacted differently toward the transplants of intact tissue than it did toward the macerated tissue as reported by Sandstrom and Kauer (1933*b*). They obtained an increase in the density of the granulocytic infiltration with an increase in the age of the donor tissue and with the length of time the tissue remained on the host. The appearance and intensity of the connective tissue reaction, on the other hand, was apparently dependent on the amount of necrotic tissue and on the number of small lymphocytes. In the grafts which contained only normal tissue there was no pronounced invasion of connective tissue. Furthermore, no such invasion occurred during the presence of many small lymphocytes. As the latter disappeared, fibroblasts entered to encapsulate the necrotic areas and tubules. No information was forthcoming to suggest that the connective tissue was related to the lymphocytes in any manner other than that of a time sequence.

There can be no doubt concerning the importance of a species specificity of the donor in accounting for the inability of tissue to grow when transplanted to a host of another species. The literature, too abundant to discuss here, established its significance and indicates that it is probably responsible for the nature of the reaction against the tissue. The question immediately arises as to the importance of the species specificity factor in causing the local reactions which appeared in the heteroplastic grafts discussed above. A comparison of the amounts of necrosis, the connective tissue reaction, and the granulocytic invasion, between homoplastic and heteroplastic grafts, revealed nothing, but did display a difference with respect to the

small lymphocytes. This difference manifested itself not in the intensity of the infiltration but in its duration. As already noted, the small lymphocytes remained in the heteroplastic grafts longer than in the homoplastic grafts—a fact of considerable importance, indicating that, at least as early as the nineteenth day, the metanephric tissue of the duck had developed certain properties peculiar to the species, i.e., had become species specific. These properties apparently caused an increased inhibition in the process of vascularization during incorporation and, therefore, a longer persistence of lymphocytes.

On the whole, however, a comparison of the reactions about the heteroplastic and homoplastic grafts revealed nothing striking. It is quite possible that intact tissue, such as used in the present experiments, does not effect the host sufficiently to call forth a more marked reaction even though the donor is of another species. This possibility is also suggested by a comparison of the results of Sandstrom (1932*a*) with those of Sandstrom and Kauer (1933*b*). Sandstrom was unable to find any conclusive evidence that properties of a specific nature, developing in either the host or donor, were responsible for his results. He secured grafts from transplants of intact metanephric tissue of variously aged ducklings, and even adult ducks, after they had remained on actively developing chick hosts for 8 days. There was not as profound an effect on the host as might be expected. On the other hand, Sandstrom and Kauer obtained some very interesting results from transplants of macerated kidney tissue of 1-week old ducklings made on the chick membrane. The mortality of the hosts was 100 per cent; death occurring within 48 hours, presumably from anaphylactoid shock. Since homoplastic transplantations of kidney tissue from hatched chicks made to the chorio-allantoic membrane caused no significant death rate, the results were interpreted to mean that a species specificity was well established in the duck tissue by hatching time.

Loeb (1921) presents information that suggests still another factor to be considered in explaining the lack of a reaction in, and about, the transplants of differentiated kidney tissue. As previously mentioned, he postulated the formation of toxins which stimulated the local reaction of the host tissue. Any graft, therefore, that could re-

move these toxins, might not elicit the reactions of the surrounding host tissue. To what extent the results of the duck kidney tissue grafts, homoplastic as well as heteroplastic, depend on this fact, is difficult to say. The nature of the normal function of the tissue might possibly permit the removal of its own toxins (metabolic products) and the complete absence of a host reaction in those grafts where function had been established explained.

The fact that metanephric tissue, equivalent in age to that of a 2-day old duckling in which a species specificity is definitely established, may grow on the chick membrane as long as the membrane affords the necessary blood supply, introduces the question of a host resistance toward heteroplastic tissue. Either the host is not species specific before hatching (i.e., it is incapable of an antagonistic reaction against the foreign transplant as such) or the transplanted tissue has become adapted to its new environment during the process of differentiation. No direct evidence has been found to indicate that the chick host is resistant to foreign tissue prior to hatching, unless the persistence of lymphocytes about the heteroplastic transplants is an indication of such a resistance. As already implied, however, the appearance of lymphocytes is a local reaction in response to poor vascularization, and is not the result of a general resistance on the part of the host. Nevertheless, the experiments are conclusive in showing that there is no sudden development of an antagonistic mechanism before hatching, such as postulated by Murphy (1914*a*, 1914*b*, 1926). The presence of normal metanephric tissue on the chorio-allantoic membrane just before hatching is a strong argument against such a possibility. Murphy apparently thought that the spleen was responsible for the defensive reaction and that this organ was not sufficiently differentiated to react against the foreign tissue until after the eighteenth day of incubation. At this time it gave rise to small lymphocytes which assumed a primary rôle in the antagonistic reaction. The results of the transplants reported here, however, are convincing in their demonstration that the lymphocytes appear in the host as early as the twelfth day of incubation. By grafts of adult chicken spleen tissue, Murphy was able to stimulate the host's spleen so that it would function in the production of small lymphocytes before the normal time. Stevenson (1917*a*, 1917*b*) and Sand-

strom (1932*b*) found that grafts of adult chicken spleen tissue caused hypertrophy of the host's spleen, which was in accord with the results of Murphy; nevertheless, they failed to find any effects on the foreign tissue transplants which were present on such hosts. Stevenson found some indications of an increase in the number of granulocytes; whereas Sandstrom found that neither lymphocytes nor granulocytes were increased.

The failure of the chick embryo to resist the grafts of metanephric tissue of the duck does not necessarily mean that under different conditions it is unable to do so. It might mean that the resistant factor, whether dependent on a single organ, such as the spleen, or on the animal as a whole, is developed gradually, allowing the transplanted tissue to become adapted to its new environment. Such an adaption would account for the unmolested growth of the duck tissue after it had acquired its species specific properties. It would be possible only in the tissue which differentiated on the chorio-allantoic membrane at the same time that the resistant factor of the host developed. Murphy (1914*a*, p. 183) stated:

> The possibility was early considered that the cells of a foreign species, if left in the embryo during the supposed gradual development of the resistant factors, might undergo an adaptation for growth in the adult. Such an adaptation I find entirely absent. The refractory condition, instead of being a gradual development, seems quickly to attain its full power.

Nevertheless, such an adaptation does not seem unreasonable in the light of the results of these experiments and the results of Erdmann (1927). She succeeded in grafting skin between different species of amphibians, but to do this she first made *in vitro* cultures of donor skin in a medium prepared from the donor species. By gradually introducing extracts prepared from the host tissues, she altered the medium until it was "homologous" to the host tissues. The explant was then implanted on the host, where it grew successfully. This apparent conditioning was a prerequisite to success, however.

SUMMARY AND CONCLUSIONS

1. The results of these experiments corroborate the earlier work of the author in showing that the capacity of duck kidney tissue for growth in either homoplastic or heteroplastic grafts is dependent on

the degree of differentiation of the donor tissue at the time of transplantation.

2. They further demonstrate that continued, unaffected growth of the duck kidney tissue in the grafts on the chorio-allantoic membrane of the chick is possible only if the transplanted tissue is capable of functioning.

3. As a corollary to the foregoing, the presence of necrotic tissue in the transplants is due either to the failure of the tissue which was already differentiated at the time of transplantation to become fully incorporated into the chorio-allantoic membrane or to the inability of the tissue which differentiated while on the host membrane to function.

4. The reaction of the host toward the transplanted tissue, in either the homoplastic or heteroplastic grafts, is local and is confined to the tissues of the host immediately surrounding the graft. It consists of infiltrations of lymphocytes, aggregations of granulocytes, and the invasion of connective tissue.

5. The lymphocytes are present in, and about, the grafts only when vascularization is inadequate, e.g., during the early stages of incorporation of the older more compact donor tissue. Their appearance is independent of the age of the host. They disappear when vascularization is sufficient.

6. Connective tissue invades only those grafts in which there is necrotic tissue. It encapsulates the necrotic areas and tubules and ultimately replaces them. The connective tissue appears as the small lymphocytes disappear, but no evidence is present to indicate any relationship other than that of a time sequence.

7. Granulocytes were present in a large percentage of the grafts, but their appearance had no consistent relationship either to the age of the host, the age of the donor, or the length of time the transplanted tissue remained on the host.

8. The donor tissue is species specific at least as early as the nineteenth day of incubation. This presence of species specificity was made apparent by a comparison of the heteroplastic and homoplastic grafts. The small lymphocytes remained in the former for a greater length of time, indicating that the transplanted tissue had developed

certain properties of a specific nature which inhibited the process of vascularization.

9. The experiments demonstrate conclusively that there is no sudden development of a general antagonistic reaction against heteroplastic transplants in the chick at any time before hatching. Although no definite information was obtained to show that the host does become resistant before hatching, the suggestion is made that, if it does develop, such a development is gradual. This would permit the differentiating embryonic donor tissue to become adapted to its new environment and continue its growth unaffected even after it had become species specific.

LITERATURE CITED

ERDMANN, R. 1927. Verwandtschaftsbeziehungen der Anurenfamilien, geprüft durch Implantationversuche gezüchteter Haut. Arch. f. Entw'mech., **112**: 739–806.

HUNT, E. A. 1932. The differentiation of chick limb buds in chorio-allantoic grafts, with special reference to the muscles. Jour. Exp. Zoöl., **62**:57–86.

LOEB, L. 1920. Heteroplastic transplantation of kidney. Jour. Med. Res., **42**: 137–46.

———. 1921. Transplantation and individuality. Biol. Bull., **40**:143–80.

———. 1926*a*. Further observations on autotransplantations and homoiotransplantations of thyroid gland in the guinea pig. Amer. Jour. Path., **2**: 99–110.

———. 1926*b*. Autotransplantation and homoiotransplantation of thyroid gland in the rat, etc. *Ibid.*, pp. 301–13.

MINOURA, T. 1921. A study of testis and ovary grafts on the hen's egg and their effects on the embryos. Jour. Exp. Zoöl., **33**:1–61.

MURPHY, J. B. 1914*a*. Studies in tissue specificity. II. The ultimate fate of mammalian tissue implanted in the chick embryo. Jour. Exp. Med., **19**:181–86.

———. 1914*b*. Factors of resistance to heteroplastic tissue grafting. III. Studies in tissue specificity. *Ibid.*, pp. 513–22.

———. 1926. The lymphocyte in resistance to tissue-grafting, malignant disease, and tuberculous infection; an experimental study. Monograph Rockefeller Inst. Med. Research, No. 21, pp. 5–30.

ROUS, P., and MURPHY, J. B. 1912. Behavior of chicken sarcoma implanted in the developing embryo. Jour. Exp. Med., **15**:119–32.

SALTYKOW, S. 1900. Über Transplantation zusammengesetzer Theile. Arch. f. Entw'mech., **9**:329–35.

SANDSTROM, C. J. 1932*a*. The growth and differentiation of duck kidney tissue on the chorio-allantoic membrane of the chick and duck. Physiol. Zoöl., **5**: 354–74.

———. 1932*b*. Heteroplastic transplants of duck kidney tissue on the chorio-allantoic membrane of the chick in conjunction with adult chicken spleen grafts. Anat. Record., **52**:69–81.

———. 1932*c*. Further observations on heteroplastic transplants of duck kidney tissue. *Ibid.*, **54**:59.

SANDSTROM, C. J., and KAUER, G. L. 1933*a*. Heteroplastic transplants of duck cartilage to the chorio-allantoic membrane of the chick. *Ibid.*, **57**:119–31.

SANDSTROM, C. J., and KAUER, J. T. 1933*b*. The growth and differentiation of macerated embryonic duck kidney tissue on the chorio-allantoic membrane of the chick. *Ibid.*, **57**:105–17.

SANDSTROM, RUTH H. 1934. The differentiation of hepatic and pancreatic tissues of the chick embryos in chorio-allantoic grafts. Physiol. Zoöl., **7**:226–46.

STEVENSON, H. N. 1917*a*. Tumor immunity in the chick embryo. Jour. Cancer Research, **2**:245–53.

———. 1917*b*. Tumor immunity in the chick embryo. *Ibid.*, pp. 449–54.

WATERMAN, A. J. 1932. The interaction between the omentum of the rabbit and transplanted organ primordia of the same species. Anat. Record, **52**:31–51.

WILLIER, B. H. 1924. The endocrine glands and the development of the chick. I. The effect of thyroid grafts. Amer. Jour. Anat., **33**:67–103.

THE EFFECT OF TOOTH INJURY ON OTHER TEETH. I. THE EFFECT OF A FRACTURE CONFINED TO ONE OR TWO INCISORS AND THEIR INVESTING TISSUES UPON THE OTHER INCISORS IN THE RAT[1]

(Thirty-one figures)

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IN A series of experimental studies on tooth development, the mode of repair and regeneration of the formative cells of the rat incisor, especially the enamel epithelium, was investigated. In the course of the study there appeared interesting and unexpected correlative abnormalities among the four incisors. The purpose of this work was to investigate the nature of this correlation. A careful survey of the literature indicates no previous similar study.

MATERIAL AND METHODS

This study is based on forty-three rats that were subjected to experimental fractures (Table I). In nine of the foregoing animals, one, and in twenty-three others, both lower incisors and their adjacent mandibular bone were fractured. In eleven cases one lower incisor and its investing bone were fractured, while the enamel epithelium of the other lower incisor was injured with a fine, surgically clean needle. The upper incisors were in no case injured. The experimental animals and their controls were selected at random from the normal rats of five different colonies and over a period of more than 2 years in order to avoid the possibility of using an abnormal strain. The age of the animals at the time of the operation ranged from 21 days to old age (at least 550 days), and their post-operative life ranged from 31 to 158 days.

A study was made of litter-mate controls of thirty-four experimental animals (Table II). A study was also made of seventy-three normal animals.

[1] This work was done under the stimulating guidance of Dr. G. W. Bartelmez, to whom the author is deeply indebted.

TABLE I

TABLE OF 43 RATS IN WHICH ONE OR BOTH LOWER INCISORS AND THEIR INVESTING TISSUES WERE INJURED BY FRACTURE

Animal No.	Age (in Days) at Operation	Killed or Died	Age (in Days) at Death	Interval (in Days) between Operation and Death	Reaction in One or More of the Uninjured Incisors		Grouping According to Eruption and Histologic Findings
					Eruption	Histologic Findings	
1	60	Killed	212	152	Retarded	Abnormal	I
44	77	Killed	200	123	Retarded	Abnormal	I
48	21	Killed	178	157	Retarded	Abnormal	I
49	21	Killed	168	147	Not measured	Abnormal	I
54	120	Killed	187	67	Retarded	Abnormal	I
57	251	Killed	353	102	Retarded	Abnormal	I
58	40	Died	87	47	Retarded	Abnormal	I
59	40	Died	100	60	Retarded	Abnormal	I
60	40	Killed	136	96	Retarded	Normal*	II
62	61	Killed	199	138	Retarded	Normal	II
63	110	Killed	141	31	Retarded	Abnormal	I
66	49	Killed	183	134	Retarded	Normal*	II
67	49	Killed	133	84	Normal	Normal	III
68	49	Died	151	102	Irregular	Abnormal	II
69	49	Killed	161	112	Retarded	Abnormal	I
70	49	Died	153	104	Retarded	Abnormal	I
72	25	Killed	169	144	Retarded	Abnormal	I
73	25	Killed	169	144	Retarded	Abnormal	I
74	25	Killed	169	144	Normal	Normal	III
76	25	Killed	169	144	Normal	Normal	III
78	59	Killed	154	95	Retarded	Abnormal	I
79	182	Killed	340	158	Irregular	Normal	III
80	59	Killed	94	35	Normal	Abnormal	II
81	59	Killed	154	95	Retarded	Abnormal	I
82	59	Killed	168	109	Retarded	Abnormal	I
83	59	Killed	154	95	Retarded	Abnormal	I
84	59	Killed	168	109	Retarded	Abnormal	I
85	59	Killed	168	109	Normal	Abnormal	II
86	59	Killed	168	109	Retarded	Abnormal	I
87	59	Killed	114	55	Retarded	Abnormal	I
88	59	Killed	154	95	Retarded	Abnormal	I
93	160	Killed	209	49	Retarded	Abnormal	I
94	450	Killed	503	53	Retarded	Abnormal	I
100	550	Died	624	74	Not measured	Abnormal	I
116	138	Killed	284	146	Retarded	Normal	II
117	199	Killed	284	85	Retarded	Normal	II
131	74	Killed	130	56	Retarded	Normal*	II
132	46	Killed	123	77	Retarded	Abnormal	I
133	46	Killed	123	77	Retarded	Normal*	II
134	51	Killed	163	112	Retarded	Abnormal	I
135	51	Died	121	70	Retarded	Normal*	II
139	52	Killed	108	56	Retarded	Abnormal	I
142	52	Killed	108	56	Retarded	Abnormal	I

* In these cases the findings were somewhat irregular but were regarded as normal since the irregularities are possibly within the normal range.

The histologic data of serial sections of incisors of one hundred ninety-eight rats that belonged to other experimental investigations conducted by the author and 753 rate-of-eruption measurements of the incisors of normal rats were also available.

The fractures were performed by means of a small wire-cutting pliers with cutting edges at right angles to the shaft (S. S. White No. 131). The fracture was confined to the imbedded portion of the in-

TABLE II

TABLE OF 14 LITTER-MATE CONTROLS TO THE EXPERIMENTAL ANIMALS OF TABLE I*

Rat No.	Litter-Mate Control to Rat	Age (in Days) When Killed
45	44	257
50	48, 49	178
61	58–63	199
63†	58–63	141
71	66–70	183
77	72–76	169
89	78, 80–84	168
90	85–88	66
91	79	107
92	79	107
119	116, 117	.....
136	134, 135	.....
137	138, 139, 142	82
141	138, 139, 142	90

* These animals showed a normal rate of eruption and a normal histologic picture in the incisors. Animals 119 and 136 showed a normal rate of eruption; their histologic picture has not yet been studied because they are still living. Animals 137 and 141 showed a normal histologic picture; their rate of eruption was not measured.

† Rat 63 was a control for 52 days, after which it was operated upon.

cisor in the region of the molars and was usually at right angles to the long axis of the tooth. No significant difference was found between an intra- or extra-oral approach. Five fractures were performed after a surgical incision and exposure of the alveolar bone. The latter method was found not to offer any advantages. In those cases where the enamel epithelium of one lower incisor was to be injured, a specially curved, fine, flexible steel pick was inserted at the gingivae and guided posteriorly within the labial alveolar periosteum to follow closely the enamel surface (Fig. 7).

The animals were fed an adequate diet, together with food scraps. Weekly (in a few cases semi-weekly) records were made of the major-

ity of the animals throughout their post-operative life in respect to their general health, the gross appearance of the incisors, the state of occlusion, and the rate of eruption. In selected cases (see rat 63) similar records were made previous to the operation. Occasionally, radiographs were taken of the heads of the living animals. The animals were weighed from time to time.

The rate of eruption was measured by making a horizontal marking with a fine file (Swiss jeweler's screw-head file) near the gingival line along the distolabial margin of the upper incisor. With an adjustable caliper provided with fine points, the distance was measured between the point where the marking crossed the distolabial margin and the point where the surface of the gingivae crossed the same margin. This distance was read in tenths of a millimeter with the aid of a stage micrometer and a dissecting microscope. A number of measurements were taken more directly with the aid of a specially constructed graduated caliper. The distance between the same points mentioned above was read 7 days later. The difference between the two readings represented the rate of eruption for that period. New marks had to be made each week in the normally erupting teeth. The same method was employed in measuring the rate of eruption of the lowers, except that here the distance read was between the lowest point of the labial aspect of the gingivae and the mid-point of the marking made across the labial surface of the tooth.

The animals were fixed with 5 per cent neutral formalin immediately after being killed or as soon as they were found dead. In every case, the head was severed and a midsagittal section was made to facilitate the radiographing of the teeth. All of the factors of radiographing were kept as constant as possible. In each case, ordinary dental occlusal films with a 5-minute exposure and a distance of 10 cm. between X-ray tube and the object were used. The further dissection consisted of separating the mandibles. In every case all the incisors were prepared for histologic study. The fixed teeth with their investing tissues were washed, decalcified in 5 per cent nitric acid followed by 5 per cent sodium sulphate, imbedded in celloidin, and stained with hematoxylin and eosin or with iron hematoxylin. In every case, the sections were mounted in serial order employing Maximow's modification of the Russian method (Romeis, 1928).

FINDINGS IN THE LIVING ANIMALS

The general health and weight of the experimental animals were, as a whole, normal and the same as that of the controls. Most of the animals showed an uneventful recovery from the fractures and the more or less temporary abscesses in the region of the injury. All but six were killed immediately before fixation.

GROSS APPEARANCE OF THE EXPOSED PORTION OF THE TEETH

Injured teeth.—The picture varied according to the interval elapsing after the operation (Figs. 22 and 24). In many cases the exposed surface of the teeth showed varying stages of discoloration. In six of the cases, where the anterior fragment was exfoliated, the posterior fragment perforated the lower border of the mandible (Fig. 21).

Uninjured teeth.—These showed no disturbance in pigment, but became elongated in 50 per cent of the animals (Figs. 6, 8, 10, 14, and 15).

The relationship between the upper and lower incisors was maintained in a functional state in 50 per cent of the animals. The latter included: (*a*) twelve animals in which the fractured incisor did not become loosened, because of ankylosis at the site of injury (Fig. 16); (*b*) two animals in which the anterior fragment was exfoliated while the posterior fragment remained in its position without growing forward. In the latter group, normal functional relationship was made possible by the fact that the adjacent lower incisor performed the function of both lower teeth (Fig. 4). In two control experiments where one lower incisor performed its own function in addition to that of the other lower tooth which had been cut off, the rate of eruption of the three remaining teeth was normal.

Although the remaining 50 per cent of the experimental animals did not show normal incisal relationship at the time of death, function had been normal during an earlier portion of the post-operative period, ranging from 2 weeks to 2 months. The incisal relationship was regarded as normal when the incisal edges showed bevels, which indicated constant wear (Figs. 2 and 6).

In 15 per cent of the cases, the uppers became elongated and curved inwardly, so that eventually the palate was perforated (Fig. 14). The experimental history of these animals was characterized

by a relatively early age at the time of operation (21–59 days) and a relatively long post-operative life (95–157 days).

Depending on the rate of eruption of the incisors and the post-operative interval, the uppers show a normal length of their exposed portions, so that their total curvature traverses an arc of about 210°, or the teeth traverse a varyingly increased arc up to and beyond 360° (Figs. 8 and 14). Similarly, the incisal bevel of the uppers shows many variations that range from the normal acute bevel seen in Figures 2 and 6 to a reverse condition seen in Figure 8.

RATE OF ERUPTION

The normal standard with which the rate of eruption of the incisors of the experimental animals was compared was based on 1,210 weekly measurements obtained in the following cases: (*a*) 114 measurements taken during the pre-operating life of the animals; (*b*) 343 measurements from litter-mate controls; and (*c*) 753 measurements of incisors of normal rats.

Injured teeth.—Eruption was found to be retarded in all cases. The retardation sometimes appeared within a week and progressed rapidly until it stopped permanently (Fig. 1).

Uninjured teeth.—The rate of eruption began to show retardation at various intervals following the beginning of the experiment. In rat 63 the eruption was reduced by one-third within the second and by one-half to three-fourths within the third post-operative week (Fig. 1). In most cases the retardation began to appear in about 4 weeks after the fracture. The degree of retardation, was, as a rule, greater in the upper than in the lower uninjured teeth.

DISSECTION FINDINGS IN THE FRACTURED MANDIBLES

Depending on the site of fracture, the post-operative period, and the condition of the anterior fragment, the mandible upon gross dissection showed characteristic disturbances. With the increase in post-operative life there was generally an increase in the size of the protuberance at the posterior end of the incisor. In two cases this protuberance was situated at the lowest level of the sigmoid notch; whereas its normal position is more anterior and inferior (Fig. 23). In six cases the posterior fragment perforated the inferior border of the mandible (Figs. 21 and 22).

RADIOGRAPHIC FINDINGS

Injured teeth.—The X-rays show clearly the site of fracture. The anterior fragment is sometimes displaced (Fig. 6). A distortion and an accumulation of calcified tissues are often found in the posterior zone (Figs. 9, 10, 14, and 16). A thickening of the alveolar bone is often seen about the site of fracture. In some animals the retained

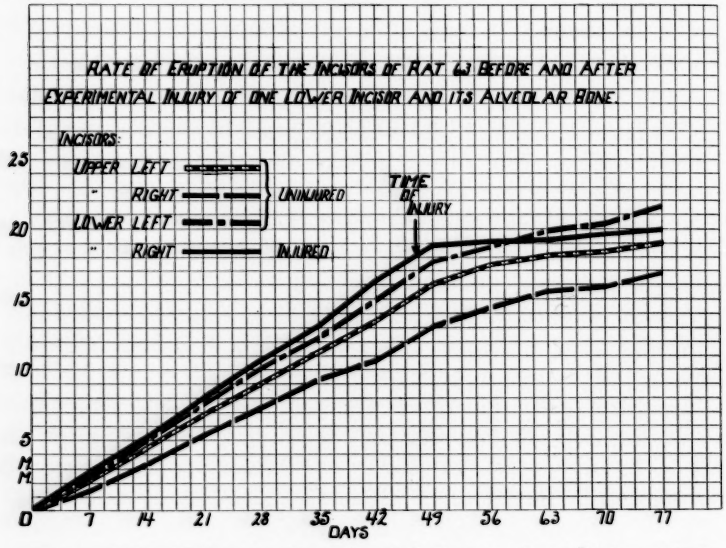

FIG. 1.—Table showing rate of eruption of the incisors of rat 63 before and after the fracture of the lower right incisor. Note that following the fracture of the latter, the eruption of all incisors was retarded.

anterior fragment is prominently smaller in its labiolingual diameter and more radiolucent than is the posterior fragment (Figs. 9 and 13).

Uninjured teeth.—The X-rays present a clear picture of the degree of elongation of the incisors and the functional condition of the incisal edges (Figs. 4, 8, 10, and 14). Even in the radiographs, a number of the uninjured teeth show following changes: (*a*) a disturbance in the contour of the labial surface; (*b*) areas of enamel hypoplasia (Figs. 10, 15, and 18).[2]

[2] For a brief description of the radiograph of the normal rat incisor, the reader is referred to Schour and van Dyke (1932).

HISTOPATHOLOGIC FINDINGS

On the basis of the extent of the eruption and histologic reaction found in the uninjured teeth, the experimental animals may be classified into three groups:

Group I comprises twenty-eight animals which showed readily recognized disturbances in both the rate of eruption and the histologic structure[3] (65 per cent).

Group II comprises eleven animals in which the disturbances were found readily only in the rate of eruption or only in the histologic structure. This group also includes animal 66, in which the reaction in both eruption and histologic structure was found to be weak (26 per cent).

Group III comprises four animals in which the uninjured teeth showed no reaction (9 per cent).

The physiologic and histopathologic findings in three representative experimental animals of Group I will be described in detail.

Rat 1.—This animal is of special interest because it is the one in which the reaction upon which this report is based was first observed. In this animal, at the age of 60 days, the enamel epithelium of the lower right incisor was injured by a fine flexible needle and the lower left incisor and its investing tissues were fractured. The radiograph taken 1 week after the injuries shows the site of fracture to be anterior to the first molar. The labial contour of the four incisors is smooth and normal. The apparent flattening of the curvatures of the teeth is a result of the angle at which the radiographs were taken (Fig. 3). There was some temporary suppuration about the gingivae of the fractured incisor. The exposed portion of this tooth became loose and was exfoliated in about 3 weeks. New tooth tissue soon grew out and came into occlusion. The rate of eruption which was measured in the upper right and in both lower incisors indicated a retardation of eruption. The animal was in good health and was killed 152 days after the operation.

Figure 24 shows the gross appearance of the head and the teeth of this animal. The incisal relationship is somewhat disturbed, but not seriously. Two streaks in which pigment is absent are seen in the

[3] For a brief description of the normal histology of the rat incisor, the reader is referred to Schour (1932).

lower right incisor. The lower left incisor shows a labial malposition.

A radiograph taken after death as a matter of routine procedure indicated an unexpected disturbance in the upper incisors which were untouched in the experimental technique (Figs. 15 and 17). Both upper and the lower right incisors show an irregular, zigzag, and sharply and repeatedly intended contour of the labial surfaces, which were perfectly smooth 1 week after the operation (Fig. 15). They also show a pulp which is confined chiefly to the posterior third of the tooth. The labiolingual diameter of the lower right incisor is about one-fourth smaller in its posterior third than in its anterior two-thirds (Fig. 18). The lower left incisor which was fractured appears to be in better condition than the rest. It recovered from the fracture. Lingual to the site of fracture there is an enlarged area of radiopaque, bonelike tissue which seems to be isolated from the rest of the mandible (Fig. 15). The angles of the incisal bevels indicate an incisal relationship which is somewhat disturbed but still functional.

The histologic pictures confirm the radiographic findings (Figs. 25 and 26). In the upper incisors, severe enamel and dentin hypoplasia is seen in the areas corresponding to those showing irregularly intended surfaces in the radiograph. The enamel epithelium is atrophic or absent in many regions. The labial alveolar bone encroaches upon the atrophied enamel epithelium and sometimes is practically in contact with the enamel (Fig. 26). The organic enamel matrix which normally extends through the posterior third of the labial surface is here found to be confined to about the posterior ninth. The cementum is thicker than normal (7 μ).

The periodontal membrane and labial alveolar periosteum are uneven in width. The dentin shows normal calcification as a whole, but there are some areas of prominent vascularization and some indentations in the lingual contour of the dentin.

The lower right incisor shows disturbances that are in principle practically the same as those seen in the uppers. In addition to the severe enamel hypoplasia and the degenerative changes in the enamel epithelium, the epithelial rests are larger and more numerous than normal and there is prominent resorption of dentin and cemen-

tum. The lower left incisor shows no enamel hypoplasia, but its enamel epithelium is reduced in the anterior third of the tooth. The cementum is thicker than normal (6 μ). Cartilage is found at the site of fracture of the lingual alevolar bone.

Rat 44.—A radiograph taken of this animal shortly before the operation shows a normal and smooth labial contour of the incisor. This animal was 77 days old at the time of the operation. The enamel epithelium of the lower right incisor was injured with a fine needle. The lower left incisor was fractured in the region of the second molar. Radiographs taken during the post-operative life indicated a development of a disturbance in the labial surface of the upper teeth. The rate of eruption of all the teeth was measured and found to be definitely retarded. The animal was in good health and was allowed to live 123 days after the fracture injury. The radiograph taken after death shows irregularities in the labial surfaces of the upper teeth (Fig. 16). The lower right incisor appears to be normal. The lower left incisor shows the seat of fracture at the level of the second molar. Its exposed portion is shortened. The posterior portion shows a folding and thickening of the calcified tissues of the labial surface. The incisal relationship appears to be functional (Fig. 16). The histologic findings in the upper left incisor are very similar to those found in the upper incisors in rat 1. The upper right incisor shows less severe disturbances, while the lower right incisor appears to be normal. The latter does not show any indications of the effect of the previous injury of its enamel epithelium.

The incisor which was fractured shows so many types of tissue reactions and disturbances that their full description must be reserved for another occasion. Some enamel has apparently still been laid down recently; but the enamel epithelium is, for the most part, entirely lacking. The investing connective tissues react to the various portions of the tooth with indifference, with apposition of bone or cementum, or with resorption. Thus, in some areas, enamel is seen to persist in its organic form and to be covered by a typical cementum with a history of lamellae and with imbedded fibers (Fig. 30). Some splinters of bone and tooth substance appear to be bathed in pus which is circumscribed by epithelium lining a cyst or a fistula.

The investing tissue contains many small circumscribed areas of calcification. The pulp is necrotic.

Rat 63.—This animal was a litter mate to rats 58–63. It was kept as a control for itself as well as for its litter mates for 10 weeks, during which the measurements showed a normal rate of eruption for all incisors (Fig. 1). The lower left incisor and its investing tissues were then fractured in the region of the first molar. The rate of eruption of all incisors was found to be retarded immediately after the operation (Fig. 1). The animal was killed soon, 31 days after the fracture, in order to see how early after the operation the histologic changes might appear. The radiograph taken after death indicates an incisal relationship which was functional, although the uppers are somewhat elongated. The upper incisors also show a considerably deeper curvature than normal (Fig. 6), indicating a delay in the flattening process that occurs normally in the rat incisor. The labial contour of the uninjured teeth appears to be normal. The lower left incisor shows a complete break in the continuity of the tooth. The anterior fragment is displaced. A large splinter of tooth substance is found labial to the incisor in the region of the second molar. The alveolar bone in the same region is considerably thickened (Fig. 6). The histologic picture shows an area of severe dentin and enamel hypoplasia in the posterior portion of the tooth, less than 2 mm. from the basal end of Hertwig's sheath in the upper and lower right incisors (Fig. 27). This may represent an early stage of the enamel hypoplasia seen more anteriorly in the other animals of longer postoperative life (Figs. 26 and 29).

Figure 28 gives a magnified view of this field. The dentin, as well as the enamel, are imperfectly formed. Large enamel globules are formed in the enamel epithelium. The lingual alveolar bone shows an abnormal spicule arrangement on the side facing the teeth.

Summary of the histopathologic findings.—Injured teeth.—A detailed report of the histologic changes associated directly with the fractures is reserved for another occasion. We shall call attention only briefly to the following features. At the seat of fracture, there is found an ankylosis of the dental tissues accompanied by various degrees of inflammatory reactions. A varying number of splinters of bone, dentin, and cementum are found scattered in the region.

Epithelial rests are found to be proliferative. Some of them form cysts; others become calcified. The pulp is necrotic in the anterior fragment and partly obliterated and dentified in the posterior fragment. Near Hertwig's sheath it has a normal appearance.

The alveolar bone at the site of fracture shows, in a few animals, cartilaginous areas. The posterior fragment shows varying degrees of accumulation and distortion of calcified dental tissues (Figs. 9, 12, and 16).

Enamel of various degrees of incomplete calcification is found to persist even in animals of longest post-operative life (Fig. 30). This enamel, however, is found to lack the normal covering of enamel epithelium. The latter in many cases has disappeared and is replaced by connective tissue or by cementum. The cementum covering such enamel in some cases takes on the form of typical primary cementum showing many lamellae and imbedded periodontal fibers (Fig. 30).

In the region immediately posterior to the fracture, the enamel epithelium undergoes atrophy and degeneration, and in many fields it is entirely absent. More posteriorly the enamel epithelium often presents severe hypoplastic areas. Hertwig's sheath is found to be functional in all cases, so that in the most posterior zones one can always find some normal enamel organ tissues.

Uninjured teeth.—The most common and constant finding is a premature atrophy of the enamel epithelium. Fields of enamel hypoplasia are common. Sometimes the dentin is hypoplastic as well (Figs. 26, 28, and 29). In fields where the enamel epithelium is lacking, the alveolar bone is sometimes seen to be almost in contact with the enamel, with only a narrow band of connective tissue intervening (Fig. 26). Hertwig's sheath is, with rare exceptions, normal in appearance. The disturbances are thus confined anterior to it. The principal tissue affected is the enamel epithelium, particularly the functional and more highly differentiated type found somewhat more anteriorly to the epithelial sheath.

DISCUSSION

For purposes of convenience and brevity, the term "ondontocytorhexic" reaction will be employed to denote a disturbance in the eruption and the histologic structure of growing teeth, caused by an

injury confined to distant teeth and their investing tissues. The term "odontocytorhexis"[4] (*ὀδόντος*, "tooth"; *κύτος*, "cell"; *ῥῆξις*, "breaking") is suggested because the experimental condition with which this report is concerned consists primarily of a breaking-up of the dental tissue cells.

Significance of the various subtypes of the odontocytorhexic reaction.—Most of the animals showed both retardation of eruption and histopathologic alterations in the teeth that were not injured experimentally. Ten showed only a retardation in eruption or only histopathologic changes. The finding of retarded eruption alone does not preclude the possibility that the histologic structure was abnormal. The histologic picture at death is a record of the tooth that formed only during the last 40 or 50 or more days, depending on the rate of eruption. In some animals, as in rat 93, enamel hypoplasia was found in the most anterior portion beyond the alveolar crest. If these animals had been killed 10 days later, this evidence would have been lost. Because of our present-day very limited knowledge of the cytology and microchemistry of the cells of the dental tissues, the finer cellular changes that are very likely associated with disturbances in growth and eruption cannot be recognized. In that event, the rate of eruption which can be measured quantitatively may be regarded as a more sensitive indicator. This is true in all but two cases (80 and 85). The absence of retarded eruption in rat 80 may be explained by the fact that it was killed early. The explanation for rat 85 is not apparent.

Involvement of Hertwig's sheath.—In view of the severe enamel and dentin hypoplasia found in the uninjured teeth of the experimental animals, it is surprising that in only exceptional cases was there any noticeable histologic disturbance in Hertwig's sheath, which is the source of the enamel epithelium and the stimulating guide to dentin formation. Such a disturbance as seen in Figure 28 indicates rather definitely that the enamel and dentin were hit at the very beginning of their formation, which means at a stage when their formative cells were very closely linked with Hertwig's sheath. The apparent immunity of the latter may be a result of our inability to recognize more

[4] We are indebted to Professor C. D. Buck for his helpful suggestions in the selection of this term.

delicate disturbances and of the high resistance of Hertwig's sheath due to its embryonic potencies. When the cells arising from the sheath move forward and differentiate and assume their functional specialization, they become more susceptible and succumb more readily to disturbing factors.

The absence of reaction in the rat molars.—The reaction that was readily observed in the incisors was not found in the molars. The latter were, in the majority of cases, already fully formed at the time of the experimental injury to the incisors. In order to check the absence of this reaction further, the lower incisors of three animals were fractured at the age of 16–21 days, when the molars were not yet erupted. In these cases, also, no reaction was found. It is possible that at the age of 16 days, the tooth germs have already developed beyond the functional stage that is sensitive to the factors concerned in the odontocytorhexic reaction. Fractures of one or two molars did not result in any reaction in the other molars or in the incisors.

The applicability of the odontocytorhexic reaction in other species.—This reaction found in the rat suggests the possibility that a severe injury to one or more tooth germs in a child may result in histopathologic changes and retarded eruption in the other tooth germs that are in a susceptible stage. This observation has not been made in the literature. It is quite conceivable that the mechanism observed in the rat may operate much less readily in the human or other species, where the formative activity of the dental tissues is slower and extends over a relatively shorter period of time than in the rat. In addition, the absence of tooth types comparable to the rat incisor in respect to the large volume of highly differentiated tissues may be a factor that is not favorable to the production of the odontocytorhexic reaction.

Injury of the enamel epithelium by needle.—The exact rôle of the needle injury in producing the odontocytorhexic reaction is not yet clear. It is possible that in the presence of the fracture in one lower incisor, the additional injury of the enamel epithelium in the other lower tooth produces a sensitizing effect which makes the reaction more pronounced. The experimental animals that showed the most strikingly irregular labial contour in the uninjured teeth (rats 1, 44,

and 49) had a fracture injury in one lower incisor and a needle injury in the other. In rat 1, the lower left incisor recovered from the fracture (Fig. 15); yet the incisors of this animal showed a strong reaction possibly because of the additional effect from the pick injury in the other tooth.

POSSIBLE BASIS FOR THE ODONTOCYTORHEXIC REACTION

The following possible mechanisms have been carefully considered, and in part tested experimentally, in order to find the primary factors in the production of the reaction: (1) functional disturbance, (2) neural disturbance, (3) general systemic disturbance, (4) endocrine disturbance, and (5) humoral disturbance.

1. *The rôle of disturbance in function.*—The factual evidence indicates that the disturbance of function is not the primary cause in the reaction.

In the experimental animals in which the reaction was produced, 50 per cent showed a practically normal incisal relationship, as indicated by the presence of the normal angles of the bevel of the incisal edges. Here, obviously, disturbance in function could not be the primary factor, since disturbed function did not exist. This percentage would have been considerably greater if a greater number of the animals had been allowed to live a shorter post-operative life.

Six animals showed a retardation of eruption which preceded a visible disturbed function, so that the latter was only secondary to the former. In animal 44, the disturbance of incisal relationship was corrected by shortening the elongated teeth, but the reaction persisted in spite of the correction of the malocclusion. In addition, in the animals in which the reaction was produced, the histologic picture indicates a blood supply that is as rich as that of the control material. If disturbance in function were the primary factor in the odontocytorhexic reaction, the prominently retarded rate of eruption of the uppers could reasonably be expected to be associated with a noticeable reduction in blood supply.

A disturbance in the occlusal and functional relationships of the molars produced by the fracture of one or two molars in three control animals resulted in no reaction other than a local one.

In animals in which disturbance in function was produced experi-

mentally by the weekly cutting-off of the exposed portions of one or more incisors for a period of 2 months or less, there was found: (*a*) a doubling of the rate of eruption of the shortened teeth; and (*b*) a normal unaltered rate of eruption of the teeth that were not shortened. These results are quite different from the effect obtained following the experimental fracture. Similar results have been observed in rabbits (Wetzel, 1927; Rink, 1929).

One control rat which was found at the age of about 60 days to have an overgrowth of the upper teeth was allowed to live for a period of over 1 year in order to see the effect of long-continued overgrowth and malocclusion of the incisors. The overgrowth was unusual and much greater than any observed in the literature. The upper left incisor continued to grow in form of a logarithmic spiral, so that it traversed the path of a number of increasingly larger circles. The upper right incisor grew into the palate, perforated it, and protruded medially through the lip and then continued its spiral course externally to the face. The lower incisors were deflected to the left side and also grew outside the oral cavity. The configuration of the incisors of this animal could not be preserved in its entirety because the distal portions were brittle and collided with the cage walls, so that parts of the exposed portions of teeth broke off from time to time. The eruption which was measured for 3 weeks in the lower incisors was faster than normal. The radiograph shows a smooth labial contour (Fig. 5), and the histologic picture shows normal dental tissues. This is an ideal control in which incisal function was severely disturbed yet the rate of eruption and histologic findings were entirely unlike those found in the odontocytorhexic reaction. There is nothing in the literature dealing with overgrowth of incisors and consequent disturbance in function that is contradictory to the foregoing.

2. *The part played by the nervous system.*—The factual evidence that is available indicates that the irritation or stimulation of the nervous fibers that may be incidental to the fracture plays very little or no part in the reaction.

The injury of nerve fibers in the fracture experiments is at most only negligible because—

1. In no case was the inferior alveolar nerve cut before it enters

the mandibular canal. Most of the fractures were made at about the region of the first molar, which is near the mental foramen.

2. The duration of the experiments assured sufficient time for adequate regeneration of the nerve fibers if they were injured.

3. None of the animals showed injuries to the lip on the side of fracture. This would occur if innervation had been disturbed, since the lips would then be bitten by the teeth because of analgesia.

4. In three control experiments in which the molars and the alveolar bones were fractured at the apical ends in a region where a portion of the inferior alveolar nerve takes its course, none of the incisors showed any changes.

It seemed advisable to test experimentally the effect of resection of the inferior alveolar nerves and the effect of the disturbance of the sympathetic innervation. A survey of the literature indicated that such experiments had been done, and so they did not need to be repeated.

Although previous workers reported negative or inconstant results (Abraham, 1899; Moral and Hosemann, 1919), Leist (1927) found that resection of the inferior alveolar nerve in the guinea pig resulted in an acceleration of incisor growth on the side of resection.

The resection of the preganglionic fibers or their paralysis produced by painting the blood-vessel wall with 5 per cent phenol also resulted in an acceleration of tooth growth (Leist, 1927; Nemtzoglou, 1926). This was explained on the basis that with the elimination of vaso-constriction, vaso-dilation has an unswayed control, so that the teeth receive a richer blood supply than normally and therefore grow faster.

Thus, the experimental findings in the literature show that the disturbance of the innervation of the rodent incisor results in an acceleration of tooth growth confined to the side involved. If, therefore, in our fracture experiments the primary factor were the disturbance of the nerve fibers, the results should have been unilateral accelerated tooth growth. In the odontocytorhexic reaction, however, there is an opposite and very different effect—namely, bilateral, retarded, and disturbed tooth growth.

3. *The general systemic condition and the odontocytorhexic reaction.*—One could conceive of the possibility that the injury and shock

incident to the fracture of the incisor and its investing tissues might lower the general health of the animal sufficiently to produce various systemic disturbances, including a retardation of the eruption of the teeth and a disturbance in the formation of the dental tissues. The evidence does not confirm this hypothesis. Unsuccessfully and partially hypophysectomized rats which were exposed to much more severe operative injuries and shock showed a normal histologic picture in their teeth (Schour and van Dyke, 1932). Three animals in which the mandible was fractured in the region of the molar roots did not produce the reaction, for reasons discussed later. The animals as a whole were in good health. Experimental rats were mated and bore litters. Their general appearance, the texture of their hair, and their size and weight were not significantly different from their litter-mate or other controls. Two of the animals developed otitis media, but a similar number of the controls did the same. The small incidence of death was not significant. The animals that showed the most severe reaction in distant teeth were not among those that died. In fact, rat 68 showed a weak reaction.

The incidence of infection in a number of the experimental animals suggests the possibility that the former may be a factor in the reaction. However, the greater number of our experimental animals showed, at best, only temporary localized infections. The severity of the reaction did not increase with the intensity of the localized infection. Four animals that yielded relatively weak reactions showed very intense infections at the site of fracture. In addition, control rats that died from pulmonary infection or control rats with severe otitis media did not show the odontocytorhexic reaction.

The evidence thus indicates that the reaction in the teeth was of a rather specific type and not part of a general systemic disturbance.

4. *The endocrines and vitamins and the odontocytorhexic reaction.*— The findings of retarded eruption and hypoplasia of enamel and dentin in our fracture experiments remind one of somewhat similar findings in disturbances of the parathyroids and the hypophysis (Erdheim, 1906; Gottlieb, 1920; Schour and van Dyke, 1932). One might, therefore, conceive of the possibility that the injury incidental to the fracture could result in sufficient disturbance in one endo-

crine or another, particularly, in the hypophysis, to produce in turn the odontocytorhexic reaction.

It is hardly necessary to state that in our fracture reaction we are not dealing with a disturbance similar to that of parathyroidectomy or hypophysectomy. A localized injury in the mandible is not likely to disturb the endocrine system. If the reaction were caused primarily by a disturbance of the endocrines incidental to the fracture injury, then one could reasonably expect a similar reaction to result from other types of fractures. However, fractures in the mandible of an intensity similar to that of at least a number of the experimental fractures, but confined to the molar region alone, or multiple fractures of the long bones did not result in changes in the incisors. In addition, unsuccessfully hypophysectomized rats, as was stated above, which were exposed to much more severe operative injuries and shock showed a normal histologic picture in their teeth (Schour and van Dyke, 1932).

Even if the fracture would result in a disturbance in the hypophysis comparable to partial hypophysectomy, this alone would not be sufficient to result in the odontocytorhexic reaction because the histologic picture of the incisor of the partially hypophysectomized rat is as a whole normal (Schour and van Dyke, 1932). That the fracture injury could result in a disturbance similar to that of complete hypophysectomy is out of question. In the latter case there results a failure in normal increase in weight and an inability to bear young. Our experimental animals showed normal weight increase and bore young. A hypophyseal disturbance in the direction of excess function is characterized by an abnormal increase in weight.

A disturbance in the parathyroids is characterized by a disturbance in calcium metabolism. The dentin of the experimental animals shows normal calcification. The experimental animals were, on the whole, in good health and showed none of the symptoms characteristic of any known type of endocrine disturbance.

Disturbances in the enamel epithelium have been associated with vitamins A, C, D, and other nutritional deficiencies. But here we again can state that the fracture experiment is not at all related to vitamin disturbances. The diet of the experimental animals was adequate. The food was offered in part in ground form and was readily

available, so that the temporary discomfort following the injury was insignificant in regard to their capacity for food intake. The experimental animals showed no symptom characteristic of any type of vitamin deficiency.

5. *The possibility of a humoral disturbance.*—Partly by process of elimination, partly because of other considerations, we have been led to entertain another possible explanation. The thought naturally occurs that, following the experimental fracture, some chemical substances may be released by the highly differentiated cells of the dental tissues that are disintegrated at the seat of injury. These substances may enter the blood stream where they circulate throughout the body, and have no effect except when coming in contact with homologous cells which are specially susceptible to these substances.

The resemblance of the odontocytorhexic reaction to sympathetic ophthalmia is obvious, and a similar explanation for the latter has been offered by Pusey (1903).

The idea that certain chemical agents have a specific and selective action on particular tissues or substances has been suggested in given instances in various fields of biology. In bacteriology we have the acid-fast Johne bacillus which causes a paratuberculous enteritis in cattle and which cannot be cultured except in the presence of dead tubercle bacilli or certain other acid-fast bacteria.

In chemotherapy, we have the specific of quinine for malaria, arsphenamine for syphilis, and chaulmoogra oil for leprosy. In serology and immunology, we have the successful use of the diphtheria antitoxin as a specific remedy of the disease. We are also dealing with many specific chemical reactions to special proteins, as in blood precipitation tests, in certain types of anaphylaxis, and in the development of specific "defensive ferments" in the Abderhalden reaction.

In physiology we have the specific action of such drugs as cocaine and curare. The specific effects of certain hormones are well known.

In genetics we have a widespread belief in the specificity of the genes with relation to heritable characters. Guyer and Smith (1918) reported the production of specific effects in form of defective eyes in the young of rabbits which, when pregnant, were injected with fowl-serum sensitized against rabbit crystalline lens.

In pathology, numerous reports dealing with the problems of specific chemical cell products have been issued (Miyagawa, 1930; Gaza, 1922; Shibata, 1929; McJunkin and Breuhaus, 1931). However, the specificity of cell products is generally regarded as contrary to present-day scientific knowledge.

The only working hypothesis which meets the experimental observations made up to the present time is that which is based on the idea of a humoral disturbance. However, the final explanation of the reaction with which this report is concerned awaits further investigation. Further experiments are now being conducted particularly to test the validity of the tentative humoral explanation.

SUMMARY

The effect of experimental injuries to the lower incisors and their investing tissues upon themselves and distant incisors has been studied in forty-three rats of 21–450 days of age and over a period of from 31 to 158 days. The experimental animals were selected at random from the normal rats of four different colonies and over a period of more than 2 years in order to avoid the possibility of using an abnormal strain. Litter-mate controls to thirty-four experimental animals were studied, in addition to seventy-three normal animals obtained from similar sources. The histologic data of serial sections of the incisors of one hundred ninety-eight rats that belonged to other experimental investigations conducted by the author were also available.

The rate of eruption was measured, and weekly records of the gross appearance of the teeth and jaws were kept throughout the duration of the experiments. A radiographic, anatomic, and histologic study was made of the teeth after death. The experimental injuries were in the nature of fracturing of one or two lower incisors and their investing tissues. In eleven cases, one lower incisor and its investing tissues were fractured while the enamel epithelium of the other lower incisor was injured with a fine needle. In no case were the upper incisors injured.

The control animals showed a normal rate of eruption and a normal radiographic and histologic picture of the dental tissues. Those of the experimental animals that were studied preceding the

injuries showed a normal rate of eruption and a normal appearance of the teeth.

In 90 per cent of the experimental animals, the result of the injuries were: (*a*) considerable retardation in eruption; and (*b*) more or less severe histopathologic changes in the dental tissues, particularly the enamel and the enamel epithelium in the injured teeth and in varying degrees in the uninjured teeth as well.

The changes were progressive and more or less permanent. In some cases they appeared within a week following the operations.

The following factors were found not to be the primary factors in the reaction: disturbances in function, in the nervous system, in the endocrines, and in the systemic condition.

The only working hypothesis which meets the observations made up to the present time is that the primary factor may be the disintegration of the differentiated dental cells in the injured teeth resulting in a release of specific substances that enter the blood stream and that influence distant homologous cells.

LITERATURE CITED

ABRAHAM, 1899. Die Durchschneidung des Nervus mandibularis. Arch. f. mikr. Anat., **54**:224–53.

ERDHEIM, J. 1906. Tetania parathyreopriva. Mitteilungen aus den Grenzgebieten, **16**:632–744.

v. GAZA, W. 1922. Die Bedeutung der Gewebszerfallstoffe (Autolysate) für das regenerative Geschehen. Arch. f. klin. Chir., **121**:378–89.

GOTTLIEB, B. 1920. Schmelzhypoplasie und Rachitis. Wiener Vierteljhrschr. f. Zahnheilkunde, Heft 2, pp. 71–96.

GUYER, M. F., and SMITH, E. A. 1918. Studies on cytolysins. I. Some prenatal effects of lens antibodies. Jour. Exp. Zoöl., **26**:65–82.

LEIST, M. 1927. Über den Einfluss des vegetativen Nervensystems auf die Zähne. I. Zeit. f. Stom., **25**:765–71.

MCJUNKIN, F. A., and BREUHAUS, H. C. 1931. Homologous liver as a stimulus to hepatic regeneration. Arch. of Path., **12**:900–908.

MIYAGAWA, Y. 1930. On the autohormone. Japan Jour. Exp. Med., **8**:185–94.

MORAL, H., and HOSEMANN, G. 1919. Über den Einfluss der Nerven auf das Wachstum der Zähne. Anat. Hefte 1. Abt., **57**:203–49.

NEMTZOGLOU, Z. 1926. Einfluss einer Halssympathikusdurchschneidung auf das Wachstum der Zähne junger Hunde. D. M. F. Zhlk., **44**:196.

PUSEY, B. 1903. Cytotoxins and sympathetic ophthalmia. Arch. of Oph., **32**: 334–38.

RINK, R. 1929. Wachstumsregeln des Kaninchenschneidezahnes während des normalen Wachstums. Vierteljhrschr. f. Zahnhlk., **45**:543–61.

ROMEIS, B. 1928. Taschenbuch der mikroskopischen Technik (München und Berlin: R. Oldenbourg), pp. 134–35.

SHIBATA, M. 1929. The influence of the parenteral injection of tooth cell substances on growth and structure of teeth. Shikwa Shimpo, Vol. **22**, No. 1.

SCHOUR, I. 1932. The teeth. In: Special cytology (edited by E. V. Cowdry; 2d. ed. [New York: Paul B. Hoeber, Inc.], Vol. 1, Sec. III, p. 68.

SCHOUR, I., and VAN DYKE, H. B. 1932. Changes in the teeth following hypophysectomy. I. Changes in the incisor of the white rat. Amer. Jour. of Anat. **50**:397–421.

WETZEL, G. 1927. Die Regulation der Nagetierschneidezähne. Arch. f. Entwklsmech., **112**:455–79.

PLATE I

FIG. 2.—Radiograph of right half of head of rat 45, which is the litter-mate control to rat 44. Natural size.

FIG. 3.—Radiograph of head of rat 1 taken 1 week after the fracture of the lower left incisor. Natural size. Note the seat of fracture in the lower incisor and the smooth labial contour of the other incisors.

FIG. 4.—Radiograph of right and left halves of rat 76, showing normal incisal edge in both upper and the right lower incisors. Natural size. The latter carried its own function and that of its fractured mate. Note in the lower left incisor the obliterations of the pulp, the backward extension of the posterior fragment, and the absence of the anterior fragment. Compare with Figure 23.

FIG. 5.—Radiograph of the head of rat 27, which was a control rat that was found to show a marked elongation of all incisors. Natural size. Functional incision was not possible. Note the smooth contour of the enamel surfaces.

FIG. 6.—Radiograph of right (above) and left (below) halves of rat 63, which was allowed to live 31 days after the fracture of its lower right incisor. Natural size. Note the acute incisal bevels, which indicate functional incisal relationship. The uppers indicate a beginning elongation. Note the seat of fracture and the displacement of the anterior fragment. Compare with Figures 1, 27, and 28.

FIG. 7.—Radiograph of a lower incisor showing the position of the flexible pick used to injure the enamel epithelium. Natural size. Note the close conformation of pick to labial curvature of tooth.

FIG. 8.—Radiograph of right half of head of rat 54, in which both lower incisors were fractured. Natural size. The post-operative life was 67 days. Note in the upper incisor the elongation and the change from the normal bevel to one of an obtuse angle. The lower incisor shows a short extra alveolar length, an abnormal incisal bevel, the seat of fracture, the obliteration of almost the entire pulp, and the accumulation of calcified tissue at its most posterior end.

PLATE I

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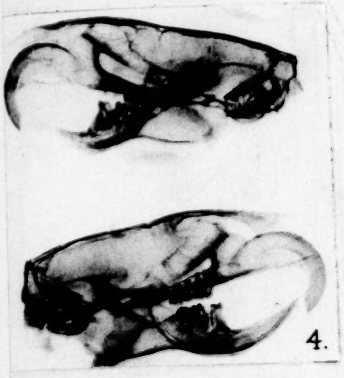
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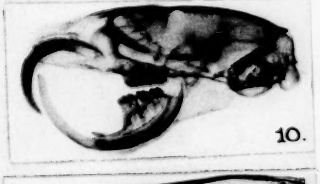
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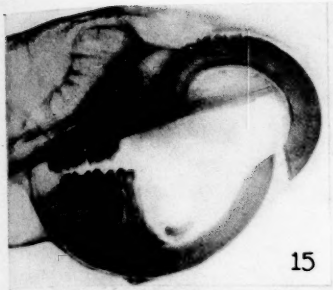
PLATE II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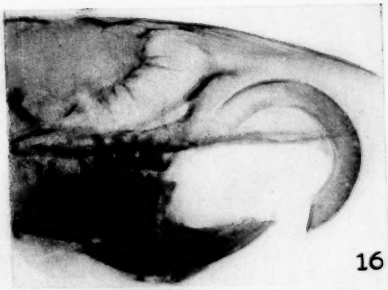
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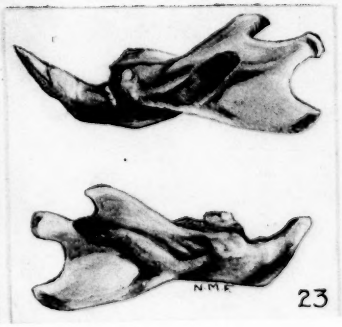
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FIG. 9.—Radiograph of left half of head of rat 59, in which the enamel epithelium of the lower right incisor was injured with a pick and the lower left incisor was fractured. Natural size. The animal lived 60 days after the operation. Note in the lower incisor the smaller labiolingual diameter and the translucent condition of the anterior fragment and the accumulation of calcified dental tissues in the posterior fragment. Compare with Figure 2 taken from a control rat.

FIG. 10.—Radiograph of right half of head of rat 49, in which the enamel epithelium of the lower right incisor was injured with a pick and the lower left incisor was fractured. Natural size. In the upper incisor note the elongation and the uneven contour of the labial surface. This animal was 21 days old at the time of the operation and was killed when 168 days old.

FIG. 11.—Radiograph of right (above) and left (below) head of rat 62, in which the enamel epithelium of the lower right incisor was injured with a pick and the lower left incisor was fractured. Natural size. Note the normal incisal edges of both upper and the lower right incisors. The latter carried its own function and that of its fractured mate. Note the abnormal relation of the posterior fragment of the lower left incisor to the rest of the mandible. The anterior fragment is lost. The post-operative life was 138 days.

FIG. 12.—Radiograph of left mandible of rat 81. Natural size. Note the retention of the fractured incisor and the accumulation of the calcified dental tissues in the posterior region. The post-operative life was 95 days.

FIG. 13.—Radiograph of right half of head of rat 84. Natural size. Note in the lower incisor, which was fractured, the radiolucent condition and small labiolingual diameter of the anterior fragment and the radiopaque condition and greater labiolingual diameter of the posterior fragment. The post-operative life was 109 days.

FIG. 14.—Radiograph of left half of head of rat 78. Natural size. Note the accumulation of dental tissues at the region posterior to the seat of fracture in the lower incisor and the perforation of the palate. The post-operative life was 95.

PLATE II

FIG. 15.—Radiograph of left half of rat 1. ×1.8. Note in the upper incisor the prominent distortion in the enamel and the partial obliteration of the pulp, and the normal incisal bevel. In the mandible note the radiopaque area anterior to the first molar. Compare with Figures 17, 18, 19, 25, and 26. The post-operative life was 152 days.

FIG. 16.—Radiograph of left half of rat 44, in which the enamel epithelium of the lower right incisor was injured with a pick and the lower left incisor was fractured. ×1.8. In the lower incisor, note the shortened extra-alveolar length, the thickening of the labial alveolar bone, the seat of fracture, and the accumulation and folding of the calcified dental tissues in the posterior region. In the

upper incisor, note the irregular contour of the enamel surface. The incisal relationship is functional, as judged by the incisal bevel. The post-operative life was 123 days. Compare with Figures 19 and 20.

FIG. 17.—Radiograph of upper right incisor of rat 1. ×1.8. Note the irregular contour of the enamel surface; the partial obliteration of the pulp; and the incisal edge, which is worn off unevenly in the mesio-distal direction. Compare with Figure 19, which is taken from a control animal.

FIG. 18.—Radiograph of lower right incisor of rat 1. ×1.8. Note the marked distortion of the posterior third of the incisor and the smaller labiolingual diameter of the latter. The pulp is partially obliterated. The incisal bevel is still functional. Compare with Figure 20, which is taken from a control animal.

FIG. 19.—Radiograph of upper right incisor of control rat 112. ×2.4. Note the normal extent of the pulp and the smooth contour of the enamel. Compare with Figures 15 and 17.

FIG. 20.—Radiograph of lower right incisor of control rat 112. ×2.4. Compare with Figure 18.

FIG. 21.—Drawing of the dissected left half of the mandible belonging to animal 66. ×1.5. Note the abnormal relation of the exposed portion of the incisor to the mandible. See Figure 22. Compare with mandible in the upper half of Figure 23.

FIG. 22.—Drawing of head of rat 66, in which, following the fracture of the lower right incisor, the anterior fragment was exfoliated and the posterior fragment perforated the lower border of the mandible. ×1.2. Note the position of the lower right incisor, which performed its own incisal function, as well as that of its malposed mate. The post-operative life was 134 days.

FIG. 23.—Drawing of left (above) and right (below) mandible of rat 76. ×1.5. Note the posterior extension of the right incisor to the sigmoid notch, following the fracture. Compare with Figure 4.

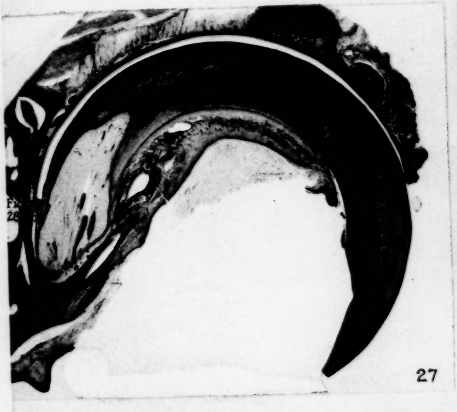
FIG. 24.—Drawing of head of rat 1, showing incisal relationship. ×1.5. Note the white areas on labial surface to lower right incisor, the slight elongation of upper right and lower left incisor, and the labial malposition of the latter.

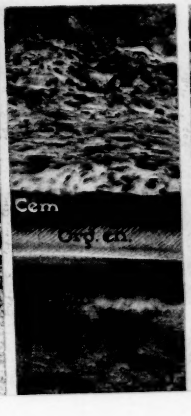
PLATE III

FIG. 25.—Photomicrograph of midsagittal section of the upper right incisor of rat 1. ×5. Note the irregular indented labial surface and the obliteration of the pulp in the anterior half of the tooth. This animal lived 152 days after the fracture of the lower incisor. Compare with Figures 3, 15, 17, 18, and 26.

FIG. 26.—Photomicrograph of area indicated in Figure 25 and magnified. ×125. Note the severe hypoplasia of enamel and dentin, *X;* the encroachment of the alveolar bone upon the enamel epithelium and the degeneration and atrophy of the latter, *Y;* and the absence of enamel and hypoplasia of dentin at *Z; Al. b.*, alveolar bone; *En. sp.*, space formerly occupied by enamel lost in decal-

PLATE III





cification; *L. a. p.*, labial alveolar periosteum. Compare with Figure 31, which is taken from a corresponding section of a control animal.

FIG. 27.—Photomicrograph of midsagittal section of the upper left incisor of rat 63. ×5. Note the deep curvature of the tooth and the area of severe enamel and dentin hypoplasia in the posterior portion. This animal was allowed to live only 31 days after the fracture of the lower right incisor. Compare with Figures 6 and 26.

FIG. 28.—Photomicrograph of hypoplastic area seen in Figure 27. ×90. Note the folding and hypoplasia of enamel and dentin, and the enamel globules (*X*) in the enamel epithelium. *Alb.*, alveolar bone; *Gan.*, ganoblasts; *L. a. p.*, labial alveolar periosteum; *odont.*, odontoblasts; *Org. en.*, organic enamel; *Pap.* epithelial papilla.

FIG. 29.—Photomicrograph of posterior portion of midsagittal section of upper right incisor of rat 49. ×45. Note the irregular folding and hypoplasia of the enamel and dentin at *X*. This animal lived 147 days after the fracture of the lower right incisor. Compare with Figure 10. *Al. b.*, alveolar bone; *Dent.*, dentin; *Gan.*, ganoblasts; *L. a. p.*, labial alveolar periosteum; *Od.*, odontoblasts; *Org. en.*, organic enamel; *Pap.*, epithelial papilla; *Pred.*, predentin.

FIG. 30.—Photomicrograph of posterior portion of midsagittal section of lower left incisor which was fractured in rat 44. ×175. Note the organic enamel matrix (*Org. en.*) that remained incompletely calcified and became covered with thick cementum (*Cem.*). The latter displaced the enamel epithelium, which is here absent. *Al. b.*, alveolar bone; *L. a. p.*, labial alveolar periosteum; *Org. en.*, organic enamel matrix; *Pred.*, predentin. Compare with Figures 16 and 31.

FIG. 31.—Photomicrograph of section from enamel epithelium of the middle portion of the upper incisor of a normal rat. ×205. *Al. b.*, alveolar bone; *En. sp.*, space formerly occupied by enamel lost in decalcification; *Gan.*, ganoblasts, *L. a. p.*, labial alveolar periosteum, *Pap.*, epithelial papillae. Compare with Figure 26.

THE QUANTITATIVE BIOLOGICAL EFFECT OF X-RAYS OF DIFFERENT EFFECTIVE WAVE-LENGTHS[1]

(One figure)

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IF LIVING matter is subjected to beams of X-rays of different wave-lengths for various periods, receiving ultimately, however, the same dosage in roentgens, will the quantitative biological effects be the same or different? This question has a practical and theoretical importance in the more critical analysis of biological phenomena under the influence of X-radiation. A number of workers have shown that when different wave-lengths are employed, the quantitative biological effects are different. Among those who hold this view are Dognon (1925) and Wintz and Rump (1926). On the other hand Wood (1924, 1925) and Packard (1927, 1929) report that X-rays of different wave lengths are equally effective biologically. This discord in observations may be due in part to the methods employed in measuring the total amount of ionization produced at widely different wave-lengths. This emphasizes very clearly the need of a standard method of describing the quality of the beams of X-rays employed.

Taylor (1931), in an attempt to find a suitable method for expressing more logically the quality of radiation, has made a comparative study of copper absorption curves for heterogeneous X-rays. It appears very clearly from his work that the radiant energy can be more logically expressed in terms of "true effective wave-length," designated so for convenience to distinguish it from the Duane (1928) effective wave-length. From his comparative study, Taylor comes to the conclusion that the method employed by him in determining the true effective length is more consistent, being less dependent upon

[1] The author is indebted to the Chemical Foundation, Inc., New York City, and to the Hendricks Research Fund (Syracuse University Medical College) for placing at his disposal the equipment necessary to the progress of this investigation.

the particular ionization chamber used in the measurement of the radiant energy. The method consists of making X-ray absorption measurements after filtration through some metal such as copper or aluminum and employing the absorption coefficients given by the slopes of tangents at various points on the plotted semilogarithmic absorption curve. The purpose of this paper is to present some observations on the biological effects of X-rays of different effective wave lengths on the gaseous metabolism of *Simocephalus vetulus*, employing distinctly the method described by Taylor of expressing the quality of the beams of X-rays used, and utilizing the constants given in a formula by Richtmyer (1921) for the determination of the true effective wave-lengths.

This work was done in the laboratories of the Department of Radiology, Syracuse University. The author wishes to express his indebtedness to Dr. A. J. King, in charge of this department, for the many courtesies received in the course of the research and particularly for the physical measurements which he made in connection with the aluminum absorption curve. Acknowledgment is also due to Mr. C. M. Schwarts for his aid in the course of the work.

MATERIALS AND METHODS

It is apparent that for a more critical study of this problem, it is important that the material employed shall consist of a single cell or of a small group of cells or of a minute organism where the question of degree of penetration and absorption does not complicate matters. In this respect, *Simocephalus vetulus* is very suitable material, for the animals, when subjected to X-irradiation, are about 0.5 mm. in length and less than this in width. The methods employed for measuring the minute quantities of oxygen consumed by the animals and the method of irradiation are the same as previously described (Obreshkove and King, 1932*a* and *b*). The animals were X-rayed immediately after they were released from the brood chamber of the mother. Since the latent period for this animal or the first appreciable change after X-radiation has not been determined and since it is suspected that this period is relatively long, the rate of oxygen consumption was not measured till 6 days after subjecting the animals to the radiant energy, when the animals became sexually

mature. This enables one to measure the degree of injury produced on the cells at a precise stage of development of the organisms. The scattering of X-rays was reduced to a minimum. The organisms were irradiated in small groups in a thin micro-culture slide without cover and in water of not more than 2 mm. in depth. The micro-culture slide rested upon a lead plate.

The X-ray apparatus employed in this work consisted of a Müller water-cooled hot-cathode type tube, having a tungsten target. The potential was supplied by a three-phase constant potential Kenotron rectified type power unit. During the irradiation period the X-ray machine was under constant observation to maintain the desired calibrated conditions.

OBSERVATIONS

The constants employed in this work for determining the true effective wave-length are those given by Richtmyer's equation:

$$\mu/\rho = 15.5\,\lambda^3 + 0.147\,.$$

Solving for wave-length

$$\lambda = \sqrt[3]{\frac{\mu/\rho - 0.147}{15.5}}\,.$$

In this equation μ is the absorption coefficient and ρ is the specific gravity of the metal employed for filtration, in this case aluminum, which has a value of 2.7. The value of μ for a particular thickness of filter is determined by obtaining the slope of the tangent to the curve at the point in question on a plotted graph representing the logarithm of the intensity as a function of the thickness of the filters employed.

Figure 1 shows the aluminum absorption curve made for 60 kilovolts and 10 milliamperes. The measurements were made with a Victoreen *r*-meter. The filters ranged in thickness from 0.42 mm. to 2.487 mm. The slope of the tangent A, for example, determines the absorption coefficient μ for filter 0.42 mm. Even at this point in the determinations it does not require much mathematical calculation to observe that the steeper the slope of the tangent at the point in question on the absorption curve, the greater is the effective

wave-length, and that linearity in the absorption curve indicates the attainment of homogeneous radiation. Making other substitutions in Richtmyer's equation for filter 0.42 mm., when $\mu = 4.25$ (as actually observed) the effective wave-length becomes 0.450 Angstrom units. This expresses the wave-length of homogeneous radiation,

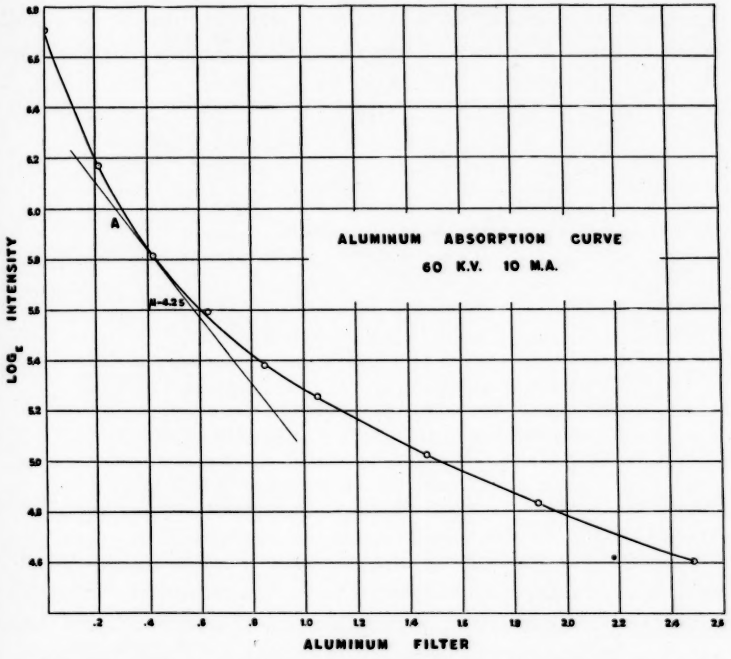

FIG. 1.—Absorption curve made with Victoreen *r*-meter, the potential was supplied by a three-phase constant potential Kenotron rectified power unit.

which would be reduced in intensity by a given filter in the same degree as the heterogeneous radiation is decreased by it.

Table I gives other aluminum absorption measurements obtained in this work and indicates the thickness of the filters employed, the intensity in roentgens per minute, the value of μ for each point in question, and the effective wave-lengths. In a previous paper (Obreshkove and King, 1932*b*) it was shown that when *Simocephalus*

vetulus is subjected to X-radiation of the same wave-length, but different doses, there is produced an injury which persists even 6 days after the irradiation. The amount of the injury is revealed by a decrease in the rate of oxygen consumption by the organism as a whole, and this is proportional to the total amount of energy which each animal absorbs. The various effective wave-lengths, as have been computed here, enable us to determine the quantitative biological effect of these different qualities of beams of X-rays on *Simocepha-*

TABLE I

A SUMMARY OF THE ALUMINUM ABSORPTION MEASUREMENTS PRODUCED AT 60 KILOVOLTS AND 10 MILLIAMPERES, EMPLOYING A THREE-PHASE CONSTANT POTENTIAL UNIT

Aluminum Filter (mm.)	Intensity Roentgens Per Minute	Absorption Coefficient (μ)	Effective Wave-Length $\lambda = \sqrt[3]{\frac{\mu/\rho - 0.147}{15.5}}$
0.42	335.6	4.250	0.450
0.63	270.0	3.310	0.411
0.847	218.7	2.685	0.380
1.05	193.8	2.375	0.361
1.47	154.0	2.080	0.342
1.897	127.0	1.890	0.329
2.487	101.2	1.740	0.317

lus vetulus when the total amount of energy to which the animals were subjected is kept constant.

Table II gives the rate of oxygen consumption of untreated individuals; this rate was determined at sexual maturity. Table III gives the results obtained with individuals which have been subjected to an intensity of X-radiation consisting of 335.6 roentgens per minute, and receiving a total dosage, as in all other experiments, of 10,068 roentgens. The effective wave-length used in this series of experiments was 0.450 Angstrom units; this was the longest wave-length employed. It is evident from a comparison of the data in these two tables that the untreated individuals show a rate of oxygen consumption more than twice that of the individuals subjected to X-irradiation. The injury produced on the individual due to X-irradiation finds expression also in the size of the animals; the untreated individuals are larger by 0.35 mm. on the average. If the

TABLE II

OXYGEN CONSUMPTION OF UNTREATED *Simocephalus vetulus* FEMALES AT SEXUAL MATURITY

(Individuals were tested in groups of four)

Average Length in Millimeters	O_2 Consumption, Cc. $\times 10^{-7}$	
	Per Group Tested Per Hour	Per Individual Per Minute
1.69	16,124	67
1.75	19,097	79
1.69	15,228	63
1.70	13,323	55
1.67	13,884	58
1.69	13,767	57
(Av.) 1.70		63

TABLE III

OXYGEN CONSUMPTION OF *Simocephalus vetulus* SEXUALLY MATURE FEMALES IRRADIATED FOR 30 MINUTES IMMEDIATELY AFTER THEIR RELEASE FROM THE BROOD CHAMBER, TO PRODUCE A TOTAL OF 10,068 ROENTGENS

Filter, 0.42 mm. aluminum; dosage, 335.6 roentgens per minute; effective wave length, 0.450 Angstrom units.

(Individuals were tested in groups of four)

Average Length in Millimeters	O_2 Consumption, Cc. $\times 10^{-7}$	
	Per Group Tested Per Hour	Per Individual Per Minute
1.33	6,218	25
1.31	5,773	24
1.38	6,662	27
1.36	5,773	24
1.38	7,166	29
1.36	5,773	24
(Av.) 1.35		25

rate of oxygen consumption per unit of protoplasm is considered, the gaseous metabolism of the untreated individuals becomes lower than actually shown, but the difference between the two sets of observations is sufficiently large to preclude any question as to the effectiveness of the wave-length employed.

Table IV gives the results obtained with the shortest effective wave-length employed, namely 0.317 Angstrom units. The biological effect produced by this quality of X-ray beam is precisely the

TABLE IV

OXYGEN CONSUMPTION OF *Simocephalus vetulus* SEXUALLY MATURE FEMALES, IRRADIATED FOR 99.5 MINUTES IMMEDIATELY AFTER THEIR RELEASE FROM THE BROOD CHAMBER TO PRODUCE A TOTAL OF 10,068 ROENTGENS

Filter, 2.487 mm. aluminum; dosage, 101.2 roentgens per minute; effective wave length, 0.317 Angstrom units.

(Individuals were tested in groups of four)

Average Length in Millimeters	O_2 Consumption, Cc. $\times 10^{-7}$	
	Per Group Tested Per Hour	Per Individual Per Minute
1.38	6,217	25
1.38	5,329	22
1.39	6,718	28
1.43	6,661	27
1.43	6,217	25
(Av.) 1.40	.....	25

same as when the longest effective wave-length in the series was employed (Table III), the rate of oxygen consumption per individual per minute for individuals of approximately the same size being in each case 25×10^{-7} cc. In our present state of knowledge it is difficult to conclude at this time whether the range of effective wave-lengths employed is sufficiently large to furnish all the necessary evidence sought for in this work. The observations recorded here do show, however, that the biological effect of X-rays of different wave-lengths within the region studied is the same. A summary of the observations obtained with other effective wave-lengths is shown in Table V. Column 4 gives the oxygen consumption per individual per minute.

The injury produced by the first two and the last two effective wave-lengths is almost precisely the same, while for the middle range of effective wave-lengths, the rate of oxygen consumption of the individuals is somewhat higher.

DISCUSSION

In the foregoing discussion it has been shown that the method of Taylor of determining and expressing the quality of X-irradiation appears logical and simple. It is based on the degree of absorption of

TABLE V

SUMMARY TABLE ON THE RATE OF OXYGEN CONSUMPTION BY *Simocephalus vetulus* SEXUALLY MATURE FEMALES, IRRADIATED FOR VARIOUS PERIODS IMMEDIATELY AFTER THEIR RELEASE WITH DIFFERENT EFFECTIVE WAVE-LENGTHS, PRODUCING IN EACH CASE A TOTAL OF ABOUT 10,068 ROENTGENS

Effective Wave-Length (1)	Irradiation in Minutes (2)	Average Body Length of Animals in Millimeters (3)	Oxygen Consumption Per Individual Per Minute Cc. $\times 10^{-7}$ (4)
0.450	30.0	1.35	25
0.411	37.3	1.38	26
0.380	46.0	1.38	32
0.361	51.9	1.47	32
0.342	65.3	1.38	33
0.329	79.2	1.39	27
0.317	99.5	1.40	25

the total energy by filters of standard material, thus furnishing knowledge of the incident radiation. The wave-lengths obtained under the conditions described here denote the wave-lengths of homogeneous radiation which would be reduced in intensity by a given filter in the same degree as the heterogeneous radiation is reduced by it (Taylor, 1931). From the results obtained in this study there can be no question but that when *Simocephalus vetulus* has absorbed a sufficient amount of the X-ray energy, injuries are produced which are measurable even 6 days after X-radiation. The degree of injury produced is dependent upon the total amount of energy absorbed rather than upon the qualities of X-rays, expressed in terms of effec-

tive wave-lengths of the type and in the manner employed in this work. In view of the observations made, it seems reasonable to assume that once injuries have been produced, the general metabolism of the cells is upset, and, in consequence, interferes with other cell activities. The disturbance may be followed by a certain degree of recovery, but it appears more logical to assume that the injury is cumulative and progressive. This conclusion finds support in the physical changes produced on the very active secretory cells lining the dorsal portion of the brood chamber (Obreshkove and King, 1932*a*). This portion of the brood chamber invaginates after irradiation and the degree of invagination increases with time. In no case has there been observed a partial or total recovery.

SUMMARY

1. The method of Taylor of determining the true effective wave-length constitutes a convenient method for expressing the quality of X-radiation. From the comparative studies of this worker the method appears logical and it is less dependent upon the type of ionization chambers employed in measuring the radiant energy.

2. The simple procedure involved in measuring the effective wave-length furnishes also a desirable method for the study of the quantitative biological effects of X-rays of different qualities.

3. When *Simocephalus vetulus* were subjected to X-rays of different effective wave-lengths, varying from 0.317 Angstrom units to 0.450 Angstrom units, the degree of injury produced on the animals was found to be dependent upon the total amount of energy absorbed rather than upon the qualities of X-rays employed.

LITERATURE CITED

DUANE, W. 1928. The measurement of effective wave lengths. Am. Jour. Roent., and Rad. Ther., **20**:241.

DOGNON, A. 1925. La mesure et l'action biologique des rayons X de différentes longeurs d'onde. Archiv. phys. Biol., **4**:87.

OBRESHKOVE, V., and KING, A. J. 1932*a*. A change in the morphology of a cladoceran effected by X-ray treatment. Phys. Zoöl., **5**:472–478.

———. 1932*b*. The effects of X-rays on the gaseous metabolism of a cladoceran. *Ibid.*, **5**:457.

PACKARD, C. 1927. Quantitative biological effects of X-rays of different wave lengths. Jour. Cancer Res., **11**:1.

———. 1929. Relation of wave length to the death rate of Drosophila eggs. *Ibid.*, **13**:87.

RICHTMYER, F. K. 1921. The laws of absorption of X-rays. Physical review, **18**:13.

TAYLOR, L. S. 1931. Absorption measurements of the X-ray general radiation. Radiology, **16**:302.

WINTZ, H., and RUMP, W. 1926. Biologische Wirkung Verschiedener Roentgenstrahlenqualitäten. Strahlentherapie, **22**:451.

WOOD, F. C. 1924. Effect on tumors of radiation of different wave lengths. Am. Jour. Roent., **12**:474.

———. 1925. Further studies in the effectiveness of different wave lengths of radiation. Radiology, **5**:199.

THE EFFECT OF CONSTANT ELECTRIC CURRENT ON THE REGENERATION OF CERTAIN HYDROIDS

(One figure)

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STUDIES on the effect of the electric current on living cells and tissues have been the source of much interesting investigation. Directly or indirectly the electric current brings about many changes within cells, some of which will be discussed later in this paper. There are, however, only a few papers dealing with the effects of constantly applied small currents on the growth of animal tissues. Mathews (1903), in connection with some other investigations, reported an inhibition of regeneration of certain hydroids where the current entered the stem. Lund (1924, and other papers), however, has examined this phenomenon in some detail in *Obelia*, and finds that treatment of the stem of *Obelia* with a constant direct current inhibits regeneration at the cathode. When the stem is placed at right angles to the path of the current the outgrowth bends toward the anode. Ingvar (1920) has found that small currents have an orienting effect on the growth of nerve fibers in tissue culture.

The work reported here deals with the effect of various current strengths on the reconstitution of the hydranth in stems of *Tubularia crocea* at Woods Hole, *Tubularia mesembryanthmum* at Naples, and *Pennaria* and *Eudendrium* at Woods Hole. I am very much indebted to Professor C. M. Child, who has given many suggestions during the course of the investigations. I wish also to thank Professor R. Dohrn for help received at the Stazione Zoologica at Naples.

MATERIAL AND METHOD

Electrical set-up.—Figure 1 shows the apparatus for treating the stems with the current. It consists essentially of a glass dish (*a*) filled with sea water which contains the animals, and two zinc-zinc

[1] National Research Fellow, 1929–31.

sulphate electrodes, which make contact with the sea water of the dish by means of two blocks of an agar–sea-water gel. The electrodes (*b* and *c*) are merely two plates of zinc immersed in dishes filled with zinc sulphate solution. The agar-sea–water bridges (*d* and *e*) were replaced frequently during the course of an experiment in order to prevent contamination by diffusion of the zinc sulphate. These bridges are placed upon blocks of agar–sea-water in the electrode dish and in the dish containing the stems.

In order to orient the stems of reconstituting *Tubularia* glass plates were covered with a thin coating of paraffin and little cham-

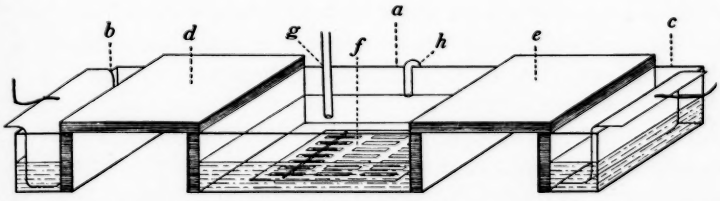

FIG. 1.—Apparatus for passing current through the stems of *Tubularia*. *a*, glass dish containing reconstituting stems; *b* and *c*, zinc-zinc sulphate electrodes; *d* and *e*, blocks of agar dissolved in sea water for conducting the current to and from dish *a;* vertical blocks conduct the current into the dishes; *f*, paraffin-covered plate on which the stems were oriented; *g*, water inlet; *h*, constant-level siphon. The agar blocks were renewed frequently.

bers cut out of the paraffin. The glass plate (*f*) was placed on the bottom of the dish which was then filled with sea water and the stems were oriented in these chambers with a glass rod placed over them to keep them in position (Fig. 1). They were usually placed alternately with the distal end facing the anode and cathode so that the normal expectation would be equal numbers of anodal and cathodal hydranths. Water was circulated through by means of inlet (*g*) and the constant-level siphon (*h*). A similar set-up was used for controls. The controls contained the agar blocks but no electrodes were used.

The current was passed through the stems continuously and its magnitude read with a milliammeter. The current strength could be varied by using different currents or by changing the level of the sea water in the experimental dish. The current strength is expressed in

$\delta = \frac{\text{microamperes}}{\text{cross section of sea water in mm.}^2}$. This value may be proportional to the amount of current which flows through the stem of *Tubularia*. The values in this paper are for the amount of current through the sea water, as it is impossible to say just how much goes through the stems.

Material.—The stems of *Tubularia* were prepared by cutting them off at the point of attachment and removing the hydranth with a few millimeters of the stem. These pieces with two cut ends were then placed in the experimental dish and the number of hydranths which emerged recorded from frequent observations. The material varied a good deal as most of the work was done in the fall and winter and the *Tubularia* at Naples were not in optimum condition at that time. Further, the temperature at Naples was rather low in some experiments so that the time of reconstitution was prolonged and the number of hydranths fewer. At Woods Hole the *Tubularia* were in better condition as indicated by a higher percentage of reconstitution. However, both of these factors, condition of material and temperature, affect the results quantitatively, and this paper presents a qualitative study of the phenomena. A more exact relationship between current density and inhibition is very interesting and will be presented in another paper.

In the following pages we shall use anodal inhibition to mean the relative inhibition of reconstitution in ends of the stems facing the positive pole. Cathodal inhibition means that fewer hydranths appear at the negative pole.

EXPERIMENTAL RESULTS

I. THE EFFECT OF THE ELECTRIC CURRENT ON THE RECONSTITUTION OF *Tubularia crocea*

Cathodal inhibition in proximal and distal halves.—We shall first consider the total number of hydranths emerged during treatment with the electric current. In the particular experiment described here, the stems were cut into halves and as there is a difference in the behavior of distal and proximal halves, we shall consider these separately. The distal and proximal halves were oriented so that half faced the positive and half faced the negative pole. There are thus

four cases, i.e., distal half with the distal end positive; distal half with the distal end negative; proximal half with the distal end positive; and proximal half with the distal end negative. When speaking of orientation we shall always refer to the distal end of a stem as pointing toward the anode or cathode. The time required for reconstitution was observed for each stem.

TABLE I

A COMPARISON OF ANODAL AND CATHODAL RECONSTITUTION IN *Tubularia crocea*

(Temperature: 16°–18° C.; Length: 15–27 mm. for halves.)

		Anodal				Cathodal				Ratio
	Current Density in δ	No. Stems Used	Total* Anodal Hydranths	Distal Anodal Hydranths		No. Stems Used	Total Cathodal Hydranths	Distal Cathodal Hydranth		Cathodal / Anodal
				No.	Per Cent			No.	Per Cent	
Distal Halves	0	85	.....	55	64	85	.....	55	64	.....
	4–5	24	20	18	75	24	15	15	62	0.7
	5–6	15	15	13	87	15	7	7	46	0.4
	6–8	31	25	20	65	31	11	11	35	0.4
	8–13	42	23	22	52	42	18	18	42	0.8
	21.6	5	4	4	.....	5	5	5	.....	1.2
Proximal Halves	0	85	.....	46	54	85	.....	46	54	.....
	4–5	24	16	14	58	24	10	10	41	0.6
	5–6	15	14	11	73	15	7	7	46	0.5
	6–8	31	25	18	58	31	8	8	26	0.3
	8–13	42	12	9	21	42	5	4	9	0.4
	21.6	5	1	1	.....	5	2	2	.....	.....

* Including proximal hydranths resulting from reversal of polarity.

Table I gives the number of hydranths present at the end of an experiment which lasted 4 days. The first column records the current density in microamperes per square millimeter; the second column the number of stems used; the third column the total number of anodal hydranths, distal and proximal; the fourth the number of distal anodal hydranths; the fifth the percentage of reconstitution of the distal anodal stems for comparison with controls. The next four columns record the same data for cathodal stems, while in the last set of figures the ratio of cathodal to anodal regeneration has been computed. The controls appear at the top of the table. Considering,

first, the distal halves, it is evident that from about 5–8 δ, a maximum effect is obtained with 0.4 as many cathodal as anodal hydranths. At 4–5 δ the effect is less, the ratio being 0.7, and astonishingly enough, increase in the strength of current (8–13 δ, also 21.6 δ) lessens the relative effect (ratio 0.8). The proximal halves show a similar relation; the maximum effect is at 6–8 δ, with a decrease with higher and lower currents.

Looking at the percentage of stems which reconstitute hydranths we find that about 64 per cent of the controls for distal halves form hydranths. Considering the cathodal inhibition at 4–5 δ reconstitution drops slightly to 62 per cent; at 5–6 δ and 6–8 δ fewer stems reconstitute, 46 per cent and 35 per cent, while at 8–13 δ the value rises to 42 per cent. Thus the absolute values for cathodal inhibition are similar to the relative values for anodal and cathodal reconstitution as expressed by the ratio.

Reconstitution at the anode is also affected as shown in Table I. From 4 to 8 δ there is a distinct increase in percentage reconstitution above the value for the controls. From 8 to 13 δ fewer hydranths develop at the anode. At first sight it would seem as if we were obtaining an acceleration of reconstitution at the anode, but in considering this phenomenon it should be pointed out that the system we are dealing with is not a simple one. The two ends of a cut stem of *Tubularia* cannot be treated independently as two isolated regions of regeneration, since it has been shown that processes taking place at one end may exert an influence over the opposite end. Thus, in general, in a system with two reconstituting regions the inhibition of one of these regions favors the other. If, for example, the distal end of a stem of *Tubularia* be inhibited the proximal reconstitution is favored. It might be expected then that inhibition at the cathode would indirectly accelerate development at the anode, without the presence of any direct influence at the anode. Whether the increase in percentage reconstitution is due to this indirect action only, or to some beneficial action of the current at the anode, or both, remains a question. It would be possible to inhibit the proximal ends of stems in some other way, without treating the distal end, and so determine how much distal stimulation is due to proximal inhibition.

Similar relations between strength of current and percentage re-

generation hold for the proximal halves (Table I). The data from the distal and proximal halves are not strictly comparable as the reconstitution of the two groups differs considerably. A larger number of distal halves regenerate as compared with proximal, and as Table II shows the distal halves regenerate in a shorter time. This shows that the capacity for reconstitution is different in the two groups and so they will necessarily be affected differently by the current.

Time required for reconstitution.—In comparing the rate of reconstitution or rather the time in hours for anodal and cathodal stems

TABLE II

CATHODAL INHIBITION IN *Tubularia crocea*. TIME REQUIRED FOR EMERGENCE OF THE HYDRANTH
(Temperature: 16°–18° C.)

Current Density δ	Time in Hours					
	Distal Halves			Proximal Halves		
	Anodal	Cathodal	Difference	Anodal	Cathodal	Difference
0	.....	45.4	.....	.....	57.6	.....
4–5	45.9	58.7	−12.8	53.7	53.2	+ 0.5
5–6	40.7	51.5	−10.8	49.9	54.3	− 4.4
6–8	44.5	53.0	− 8.5	47.1	65.2	−18.1
8–13	45.8	58.7	−12.9	50.5	60.0	− 9.5
21.6	61.0	56.0	+ 5.0	66.0	44.0	+22.0

to form hydranths, equal numbers of stems were used for the calculations. There was considerable variation in the length of time for complete development and in the case of the cathodal hydranths those which normally would have taken a long time to develop failed to reconstitute in the current.

Table II gives the average times for reconstitution for anodal and cathodal hydranths. Considering the distal halves, the retardation amounts to 12.8, 10.8, 8.5, 12.9 hours for the cathodal stems. At 21.6 δ anodal retardation occurs, and the anodal stems are retarded by 5 hours.

The cathodal proximal halves show a similar retardation, the values being 4.4, 18.1, and 9.5 hours. At 21.6 δ anodal inhibition sets in, causing a difference of 22 hours.

"*Reversed polarity*."—When a stem of *Tubularia* is sectioned in two places ordinarily a hydranth develops at the distal cut end, and, depending on the physiological condition of the material, a hydranth may appear later at the proximal cut end. In the material used in these experiments the proximal hydranth developed in only a few cases in controls which were not treated with the current. Therefore, these stems exhibited a rather strong polarity. Since early workers have shown that inhibition of the distal end stimulates the proximal hydranth to develop, it is not surprising that in these experiments hydranths often appeared at the proximal end when this end was positive. Inhibition of the negative end may be said to account for such hydranths and the phenomenon is here termed "reversed polarity." Whenever a hydranth appears at the proximal end before the distal hydranth has emerged the stem is said to exhibit this "reversed polarity." This term does not imply that the organic polarity of the stem has been altered, but is only used to describe those cases in which a proximal hydranth emerges and the distal hydranth is either suppressed or greatly retarded. The polarity of the stem of *Tubularia* is quantitative in nature in that while a hydranth is reconstituted at both ends, the proximal hydranth is smaller and takes a longer time to develop. When the current inhibits the reconstitution of the distal hydranth at the cathode, the proximal end often reconstitutes first. The polarity relations are thus the reverse of the controls where the distal hydranth reconstitutes first and the proximal one much later or not at all. In the experiments dealing with the treatment of distal and proximal halves, *no proximal* hydranths developed in controls during the time of the experiment. In the other experiments a few proximal hydranths emerged in controls.

Table III records the cases of "reversed polarity" in the experiments related above. Column 2 gives the total number of stems treated, Column 3 the number of cathodal hydranths which developed, Column 4 the number of stems which should have developed a cathodal hydranth *at the distal end*, but instead formed an anodal hydranth at the proximal end. Column 5 records the number of stems which formed a hydranth at the cathodal proximal end instead of the anodal distal end.

Looking at the distal halves we find ten cases of "reversed polar-

ity" from the cathode to the anode and no cases in the opposite direction. Similarly fifteen proximal halves show "reversed polarity" when facing the cathode and only one toward the anode. The maximum effect is again at 6–8 δ, five cases in the distal halves and seven cases in the proximal halves. In the proximal halves at this current density almost as many show reversed as normal polarity, seven compared with eight.

Cathodal inhibition in whole stems.—Some entire stems were treated with two current strengths and the results are found in Table IV.

TABLE III

"REVERSED POLARITY" IN *Tubularia*

(−to+ means that a stem with its distal end facing the negative pole developed a hydranth at the proximal or positive end. Temperature: 16°–18° C.)

Current Density in δ	Distal Halves				Proximal Halves			
	No. Cut	Cathodal Hydranths	"Reversed Polarity"		No. Cut	Cathodal Hydranths	"Reversed Polarity"	
			−to+	+to−			−to+	+to−
4–5	24	15	2	0	24	10	2	0
5–6	15	7	2	0	15	7	3	0
6–8	31	11	5	0	31	8	7	0
8–21.6	47	13	1	0	42	7	3	1
Total No.	.....	46	10	0	.....	32	15	1
Time in hours	.....	.....	58.0	.....	.....	.....	65.2	81.5

At 9.3 δ there is a decided polar effect with negative inhibition. At 11.8 δ equal numbers of anodal and cathodal hydranths developed, although one of the anodal hydranths is a case of "reversed polarity." Comparing times for development at 11.6 δ the anodal pieces take much longer than the cathodal, although both show inhibition compared with controls.

Anodal inhibition.—The data from higher current densities in the above experiments show that cathodal inhibition falls off and at 11.6 δ (Table IV) anodal inhibition sets in. Only a few experiments exhibiting anodal inhibition were performed on *Tubularia crocea*. Later, this phenomenon was examined more in detail in *Tubularia*

mesembryanthmum at Naples. Table V gives results of two experiments on *Tubularia crocea*. At a current density of 11.3 δ, forty stems formed nineteen hydranths at the cathode and six at the anode. Five of the cathodal hydranths were cases of proximal reconstitution, i.e., "reversed polarity" from anode to cathode. At the

TABLE IV

EFFECT OF 9.3 δ AND 11.6 δ ON RECONSTITUTION OF WHOLE STEMS OF *Tubularia crocea*
(Temperature: 16°–18° C.)

Current Density	Stems	No. Cut	No. Hydranths	Time in Hours	"Reversed Polarity"
9.3	Anodal	10	7	61	3
	Cathodal	10	3	67	0
	Controls	10	9	50.1	0
11.6	Anodal	8	5	76	1
	Cathodal	8	6	57	0
	Controls	10	10	44.2	0

TABLE V

ANODAL INHIBITION IN *Tubularia crocea*
(Temperature: 12°–14° C. R.P. means "reversed polarity.")

Current Density	No. Cut	Anodal Hydranths*	Cathodal Hydranths*	R.P. +to−	R.P. −to+	Time in Hours		
						Anodal†	Cathodal†	R.P. +to−
11.3 δ	40	6	19	5	1	120	91	153
27.2 δ	20	4	8	.....	.....	.....	.....	.....

* Including hydranths resulting from R.P.
† Not including hydranths resulting from R. P.

end of this experiment sixteen stems had developed a hydranth at the cathodal ends only, four at the anodal ends, only two possessed hydranths at both ends, and four were developing hydranths at the cathode. Of the four which were developing, two were cases of "reversed polarity."

These experiments show quite conclusively an inhibition of hydranth formation at the anode. Not only did fewer hydranths emerge

at the anode but those which did were distinctly smaller than at the cathode.

Since the temperature was much lower in these experiments as compared with previous ones, the hydranths reconstituted much more slowly. In Table V the cathodal hydranths reconstituted in 91 hours, while the anodal ones took 120 hours. Those exhibiting reversed polarity emerged still later, 153 hours.

II. THE EFFECT OF THE ELECTRIC CURRENT ON THE RECONSTITUTION OF *Tubularia mesembryanthmum*

The preliminary experiments with *Tubularia crocea* showed that for most of the current densities used an inhibition of hydranth formation was obtained at the negative pole, but the strongest currents caused inhibition at the positive pole. Since there were only a few experiments showing anodal inhibition, and since only a narrow range of current densities was tried, it was necessary to examine more in detail this anodal inhibition. In speaking of anodal inhibition we mean that fewer hydranths emerged on the positive side as compared with the negative side, the normal expectation being equal numbers. In most of the experiments on *Tubularia mesembryanthmum* at Naples relatively strong currents were used.

Table VI summarizes the data obtained with *Tubularia* at Naples. These experiments differ from those on *Tubularia crocea* in a few respects. In the first place, the entire stem was used instead of halves. Second, the total number of hydranths which have reconstituted after a period of time is recorded, and so the times for reconstitution are not calculated. Third, there was a lower percentage of reconstitution in this form at the low temperatures at which I was working. Fourth, controls are wanting in several experiments because of lack of material.

The total number of anodal hydranths which are present at the end of an experiment is recorded in Column 3 and the cathodal hydranths in Column 4 of the table. In Column 2 are the total numbers used for the experiment, and in each experiment half of these stems were oriented with the distal end pointing toward the positive pole and half with the distal end negative. The ratio of cathodal to anodal hydranths is found in Column 5. Comparing the effect of different

current densities it is interesting to note that a marked cathodal inhibition occurs at 7δ, which was about the same value found for *Tubularia crocea*. Sixty stems reconstituted twenty-one anodal and nine cathodal hydranths, giving a ratio of 0.42. Three of the twenty-one anodal hydranths were proximal, indicating a "reversal of polarity" from the negative distal end to the positive proximal end. Only

TABLE VI

EFFECT OF A CONSTANT ELECTRIC CURRENT ON THE RECONSTITUTION OF *Tubularia mesembryanthmum*
(Temperature: 13°–19° C.)

Current Strength δ	Number of Stems	Number of Hydranths Reconstitute		Ratio −/+	Reversed Polarity		Tropism	Controls	
		Pos.	Neg.		+to−	−to+		Number Cut	Number Reconstituted
2.3	80	11	10	0.90	0	0	—	.....	.....
5.1	200	41	40	0.97	0	0	*	.....	.....
7.0	60	21	9	0.42	0	3	—	.....	.....
10.3	180	38	41	1.09	0	0	—	.....	.....
42.8	120	32	40	1.25	0	0	—	.....	.....
62.8	382	50	140	2.80	28	1	—	130	75
77.1	80	21	39	1.80	9	2	—	.....	.....
78.6	120	18	31	1.72	.....	.....	*	40	26
85.6	160	18	46	2.55	.....	.....	—	20	10
90.4	40	0	2	?	.....	.....	*	20	16

* Not observed.

two current strengths less than 7 δ were employed, and they show no significant effects.

As the current strength is increased some very interesting changes occur. Even at 10.3 δ an indication of anodal inhibition is found, while at 42.8 δ, and especially at 62.8 δ, unmistakable inhibition is occurring at the positive pole. At 62.8 δ almost three times as many cathodal hydranths emerge as compared with the anodal hydranths. Further, twenty-eight of the one hundred forty cathodal hydranths were proximal, i.e., show "reversed polarity" from the positive distal end to the negative proximal end.

The results from higher current strengths show that there is a wide

range of anodal inhibition as compared with cathodal inhibition, for it is obtained from 42.8 δ to 90.4 δ.

Effects of the current on the structure of the hydranths.—With current densities of 42.8 δ or above there is a decided difference in the appearance of anodal and cathodal hydranths. Those which emerge at the positive pole are always much smaller and often deformed or lacking in parts. Thus, for example, one or both sets of tentacles may be absent and the body of the hydranth misshapen.

The difference in size can be conveniently measured in the primordium within the perisarc, and this was done in a few cases. The

TABLE VII

COMPARISON OF LENGTH OF ANODAL, CATHODAL, AND CONTROL PRIMORDIA
(Current 62.8 δ.)

No.	Anodal	Cathodal	Control
1	80	120	170
2	63	130	160
3	.....	130	180
4	.....	.....	130
5	.....	.....	145

following measurements were made on some stems which had been treated with 62.8 δ. The units are arbitrary, and although the data are incomplete, it will be seen that the cathodal hydranths are intermediate between anodal and control hydranths (Table VII).

In this experiment five stems were placed distal end positive, five distal end negative, and ten were used for controls. Forty-five hours after the experiment began, the length of the primordium was measured with an ocular micrometer in those stems which were reconstituting. Of the five anodal stems two primordia gave eighty and sixty-three units respectively; of the five cathodal stems three measured 120, 130, 130. The measurements of five controls are in Table VII. A sixth had already emerged so could not be measured. The two anodal hydranths died, while the three cathodal and five controls emerged.

Effects of the current on the orientation of the hydranths.—In addition to the effect of size and number there is an effect on the orienta-

TABLE VIII

"ELECTRICAL POLARIZATION" OF *Tubularia* STEMS

(Current density 62.8 δ. The signs in Column 3 refer to the polarity of the applied current at the distal end. Thus in the first two the distal end faced the positive pole. The sign of the reading is of the distal end of the stem and the magnitude in microvolts. Stems 25 mm. long.)

Colony	No.	Applied Current	Potential Difference		Sign of Distal End (20 Hours)
			After Cutting	25 Minutes	
A	1	+	−250	+310	+260
	2	+	− 70	+250	+240
	3	−	−140	−220	−390
	4	−	−180	−300	−340
	5	+	−190	+280	+150
	6	+	−200	.....	+150
	7	−	−110	−210	−230
	8	−	−130	−230	−340
	9	+	−670	−160	+210
	10	+	−180	+360	+160
	11	−	− 60	−250	−460
	12	−	−350	−360	−250
B	13	+	−200	+110	+230
	14	+	−160	+ 80	+160
	15	−	−260	−160	−230
	16	−	−130	−140	−210
	17	+	− 80	.....	− 60
	18	+	−200	+130	.....
	19	−	.....	−230	−310
	20	−	.....	−170	−520
C	21	+	− 90	+190	+ 50
	22	+	−130	+170	+390
	23	−	−220	−250	−330
	24	−	−320	−480	−350
D	25	+	?	+170	+580
	26	+	+ 60	+450	+380
	27	−	+150	−260	− 90
	28	−	+100	−260	−400
E	29	+	0	+250	+560
	30	+	0	+200	+270
	31	−	0	−170	−270
	32	−	0	−250	−270
F	33	+	+100	+240	+250
	34	+	0	+220	+110
	35	−	+150	−160	−110
	36	−	− 30	−180	− 70
G	37	+	+150	+360	+120
	38	+	−120	+220	+310
	39	−	+150	−290	−270
	40	−	+150	−260	−180

tion of the hydranth in the current. The anodal hydranths are found to bend around so as to face the negative pole, while the cathodal hydranths grow straight out toward the negative pole.

If stems with attached hydranths be placed at right angles to the path of the current, they will bend toward the negative pole. This reaction depends on the age of the individual, young stems reacting quickly, old stems slowly, if at all. Of course, the strength of the current also determines the response. In Table VI the response of the hydranth is indicated in column headed "tropism." A negative sign indicates that the hydranth bends toward the negative pole.

III. THE EFFECT OF THE ELECTRIC CURRENT UPON THE POTENTIAL DIFFERENCES BETWEEN THE DISTAL AND PROXIMAL ENDS OF *Tubularia*

After the current has been acting for some time the stems become polarized and a potential difference can easily be measured by means of a potentiometric setup. The magnitude of the potential difference is of the same order as that which can be measured in untreated stems, and the polarity is opposite to the impressed potential difference through the stem. That is, the polarization current flows in the opposite direction to the current which is sent through the stem.

The stems become polarized very quickly at 62.8 δ. Table VIII gives the potential differences in forty stems taken from seven colonies. The third column indicates the direction of the applied current; thus in No. 1 and 2 the current entered the distal end; in No. 3 and 4 the current left the distal end. The fourth column records the potential differences after cutting and before treatment. They are variable, the majority showing the distal end negative. In Column 5 the potential differences, 25 minutes after the current was turned on, show the stems to be completely polarized. After 20 hours there is little difference, the magnitude of the potential difference being about the same as that at 25 minutes. Isolated observations show that a few minutes' treatment is sufficient to polarize the stems.

The electrical polarity thus impressed persists after removal of the applied current. Table IX records the potential differences in twenty *Tubularia* stems which had been treated for 36 hours and then removed to a control dish.

With the exception of four stems the impressed polarity persists.

These four stems had faced the anode and the distal end became positive. As Table VIII shows, the distal end is usually negative in untreated stems, and therefore it is not surprising that some of the stems should return to the negative condition.

In another experiment eight stems were treated with a current of 10.2 δ and the potential differences recorded from time to time. After 4 hours the potential differences of stems with the distal end cathodal were −790, −985, −740, and −1320 microvolts. The corresponding stems oriented with the distal end toward the anode gave −460, −630, −600, −230 microvolts. The sign of the reading refers to the

TABLE IX

ELECTRICAL DIFFERENCES IN POTENTIAL 1–2 HOURS AFTER TREATMENT WITH ELECTRIC CURRENT
(Current density 62.8 δ. Readings in microvolts.)

Distal End toward Anode	Distal End toward Cathode	Distal End toward Anode	Distal End toward Cathode
+230	−250	+120	−250
+360	−330	−310	− 90
+200	−290	+120	−170
+ 80	−210	0	−440
−150	−220	−150	−340

distal end. In this experiment the anodal hydranths emerged before the cathodal hydranths.

A current of 10.2 δ does not cause the distal end to become positive when the distal end is oriented toward the anode, but does decrease the magnitude of the potential difference below that of stems with the distal end facing the cathode.

IV. THE EFFECT OF THE CURRENT ON *Eudendrium* AND *Pennaria*

Similar experiments were carried out with *Pennaria* and *Eudendrium*, and the results are extremely interesting. These hydroids are branched colonial forms and for the experiments the main stem of a colony was used. The side branches were removed and stems of various lengths were treated continuously with a constant direct current in the same manner as *Tubularia*. Regeneration differs somewhat in *Pennaria* and *Eudendrium*. In the former, outgrowths appear at the apical and basal ends of a stem, and the apical growth

usually gives rise to a hydranth. In *Eudendrium*, however, it was the basal growth which, in my experiments, usually differentiated into a hydranth. Both forms differ from *Tubularia* in that there is an outgrowth of the coenosarc which forms the hydranth, while in *Tubularia* the hydranth differentiates in situ within the perisarc.

TABLE X

COMPARISON OF ANODAL AND CATHODAL OUTGROWTHS IN *Eudendrium*
(Temperature: 21° C.)

Current Density δ	No. Stems	Length mm.	Outgrowths at about 48 Hours	
			Anodal	Cathodal
2.3*	20	20	10	9
7.0*	20	10–20	10	9
7.5	40	20–25	16	9
15.1	20	15–20	11	0
25.3	40	25	29	0
25.3	60	25	42	4
32.8	40	20	38	7
50.5	40	15–20	31	0
50.5	40	20	35	0
50.5	60	15–20	41	0
62.8*	40	20	18	0
62.8*	20	20–25	10	0
62.8*	20	10	13	0
62.8*	20	15	12	0
90.9	40	15–25	20	0
202.0	40	20–25	19	0

* *Eudendrium* at Naples.

Cathodal inhibition in Eudendrium.—The results with *Eudendrium* are very striking (Table X). With the exception of five experiments, no cathodal outgrowths were obtained. The anodal outgrowths are both basal and apical, and hydranths form from some of the outgrowths. Most of these experiments were performed on *Eudendrium* at Woods Hole, with the exception of 2.3, 7.0, and 62.8 δ in which *Eudendrium* at Naples was used.

The effect of varying the time of treatment.—In one experiment eighty stems of *Eudendrium* were selected and divided into four lots. Lot *E* was treated continuously, *B* for 43 hours and then returned to the control dish, and *A* for 19 hours, while *C* served as a control.

The total number of growths is recorded in Table XI. With shorter exposure relatively more cathodal outgrowths appear.

Reversibility of the inhibiting effect.—Since with shorter exposure more outgrowths form, the inhibiting effect is not due to a radical injury to the cathodal end of a stem. To throw further light on the nature of the inhibiting effect of the current, the current was reversed during the progress of an experiment.

Forty stems of *Eudendrium* about 15–20 mm. long were treated with 50.5 δ for 48 hours. At the end of this time there were thirty-one anodal outgrowths and none at the cathode. The current was

TABLE XI

EFFECT OF VARYING THE TIME OF EXPOSURE OF *Eudendrium* TO AN ELECTRIC CURRENT

(Temperature: 16° C. Stems 20–25 mm. long. Current density 62.8 δ)

Lot	No.	Treatment	Outgrowths 5 Days	
			Anodal	Cathodal
E	20	Continuous	3	0
B	20	43 hours	10	3
A	20	19 hours	7	4
C	20	control	10	9

then reversed, and 24 hours later many of the outgrowths had disappeared and a few of the inhibited ends showed outgrowths. Forty-eight hours later all of the formerly anodal, now cathodal, outgrowths had disappeared, while nine of the formerly cathodal, now anodal, ends had begun to regenerate.

In another experiment sixty pieces of *Eudendrium* were treated 24 hours with the current (50.5 δ) and thirty-five anodal ends began to regenerate. Current was then reversed. Twenty-four hours later only nineteen of the thirty-five outgrowths remained, while the formerly inhibited ends were beginning to regenerate.

In still another experiment forty pieces, 20–25 mm. long, were treated for 18 hours with a current of 32.8 δ. The condition of the stems is indicated by Table XII (8/14/30). There were in all thirty-eight anodal and seven cathodal outgrowths. The current was re-

versed, and on 8/15/30 the thirty-eight anodal regenerating ends were reduced to thirty-five cathodal, and the seven cathodal had increased to twenty-two anodal. At 8/16/30 the original thirty-eight had decreased to twenty-seven and on 8/17/30 to fourteen. The

TABLE XII

REVERSAL OF CURRENT ON REGENERATION OF *Eudendrium*

(Current density 32.8 δ. Temperature: 21° C. Stems 20–25 mm. long.)

Date Begun: 8–13–30 3 P.M.	Electrical Polarity	Polarity of Stem	No. of Outgrowths	No. of Hydranths	Total
8/14/30 9 A.M.	+	apical	19	.....	38
		basal	19	.....	
Current reversed	−	apical	4	.....	7
		basal	3	.....	
8/15/30 9 A.M.	−	apical	4	14	35
		basal	15	2	
	+	apical	4	.....	22
		basal	18	.....	
8/16/30 9 A.M.	−	apical	6	9	27
		basal	7	5	
	+	apical	9	1	29
		basal	19	0	
8/17/30 9 A.M.	−	apical	6	1	14
		basal	4	3	
	+	apical	0	7	26
		basal	6	13	

original seven increased to twenty-nine and on 8/17/30 twenty of these growths differentiated into hydranths.

Thus, reversal of the direction of the current completely reverses the effect. The action of the current after reversal is not immediate, as shown by comparing the condition of the stems on 8/14/30 with those on 8/15/30. Considering the nineteen anodal apical out-

growths fourteen differentiated into hydranths and four remained as growths, while only one disintegrated.

Likewise, the nineteen anodal basal outgrowths after reversal of the current formed two hydranths, while fifteen remained as outgrowths, and two disintegrated. However, on 8/16/30 of the fourteen hydranths at the apical ends only nine remained, and on 8/17/30 all but one disintegrated. Some of the basal ends continue to differentiate until 8/16/30, forming five hydranths. Of the nineteen regenerating ends only twelve remain (seven outgrowths plus five hydranths). On 8/17/30 only seven basal growths are left (four outgrowths plus three hydranths).

The reversal of the current affects the cathodal outgrowths, especially the basal ones, very quickly. At 8/14/30 there are only three such outgrowths, but upon reversal this number increases to eighteen in 24 hours. The number of cathodal apical outgrowths does not increase during this period, but during the next 24 hours increases from four to nine, and at the end of the experiment there were seven hydranths.

Thus, while before reversal of the current the ratio of regeneration in the stems stands thirty-eight anodal to seven cathodal, after reversal at the end of the experiment the ratio of the same stems becomes fourteen cathodal to twenty-six anodal.

The effect of the current on the adult colony of Eudendrium.—It was observed during the experiments that the hydranths, when at right angles to the path of the current, would bend toward the negative pole. This was due to contraction at the cathode, since the hydranth returned to its original position when the current was broken.

This bending was examined more in detail, and it was found that the tentacles of the hydranth also responded to the current by contraction at the cathode.

To cite an experiment: a colony of *Eudendrium* was placed so that the current when made would pass at right angles to the hydranths. The cross-section of the sea water and the current was adjusted so as to give 101 δ. The hydranths were observed under a binocular while the current was made. The tentacles, which are ordinarily extended, contracted on the cathodal side but remained extended on the anodal side. With continued action of the current the

entire hydranth slowly turned until the hydranth was almost in line with the current. The effect of breaking the current was not studied. The important point here is that in *Eudendrium* contraction occurs at the cathode and that inhibition of regeneration also occurs at the cathode.

Anodal inhibition in Pennaria.—We shall consider first the outgrowths from the two cut ends. In all experiments the stems were oriented so that the distal ends alternately faced the anode and cathode. The results of various current strengths are tabulated in

TABLE XIII

EFFECT OF AN ELECTRIC CURRENT ON THE REGENERATION OF *Pennaria*

(Temperature: 21° C.)

Exp. No.	Current Density δ	No. Stems	Length in mm.	Anodal Outgrowths	Cathodal Outgrowths	Ratio: Cathodal / Anodal
1	7.5	56	20–25	35	31	.8
2	12.6	80	15–25	67	71	1.0
3	15.1	40	15–25	14	33	2.3
4	32.8	40	20–25	8	23	2.8
5	50.5	60	15–20	12	32	2.8
6	101.0	80	.....	0	6	.....
7	101.0	40	.....	0	9	.....

Table XIII. With increasing current densities the number of anodal outgrowths becomes less and less until at 101 δ no regeneration takes place at the anode.

At 101 δ there also occurs a marked inhibition of the cathodal ends as compared with controls. In Experiment 7, for example, of forty stems used only four apical and five basal ends showed signs of regeneration. At the same time twenty-four controls gave nineteen hydranths and eighteen outgrowths.

The reversibility of the inhibiting effect was tested by reversing the current through the dish after 18 hours of treatment. Referring to Table XIV, forty stems of *Pennaria*, 20–25 mm. long, were treated with 32.8 δ for 18 hours with one anodal growth and thirty-seven cathodal outgrowths. After reversal of the current the thirty-seven outgrowths decreased to thirty-six in 24 hours, to twenty-eight in 48

hours, and eight at the end of the experiment. The single outgrowth increased to twenty at 24 hours, twenty-five at 48 hours, and at the end of the experiment there were twenty-two growths (twelve hydranths plus ten outgrowths).

TABLE XIV

EFFECT OF REVERSING THE CURRENT AFTER CATHODAL REGENERATION OF *Pennaria* BEGINS

(Current 32.8e. Temperature: 21° C.)

Date Begun: 8–13–30 3 P.M.	Electrical Polarity	Polarity of Stem	Number of Outgrowths	Number of Hydranths	Total Regeneration
8/14/30 9 A.M.	+	apical	1	0	1
		basal	0	0	
Current reversed	−	apical	19	0	37
		basal	18	0	
8/15/30 9 A.M.	−	apical	10	0	20
		basal	10	0	
	+	apical	18	0	36
		basal	18	0	
8/16/30 9 A.M.	−	apical	6	5	25
		basal	12	2	
	+	apical	17	.....	28
		basal	11	.....	
8/17/30 9 A.M.	−	apical	4	7	22
		basal	6	5	
	+	apical	5	1	8
		basal	2	0	

The action of the electric current on the contraction of the tentacles and the bases of the hydranth.—If a hydranth be placed at right angles to the path of the current, the tentacles will contract on the side facing the anode, while those facing the cathode will remain extended. At 15.1 δ this condition may last for a long time, while the current is flowing. At 101 δ the action is more vigorous and the tentacles on

the anodal side shrink down to the body of the hydranth. In this same current the hydranths will bend toward the anode, indicating a contraction on that side.

DISCUSSION

Lund (1924) has reported cathodal inhibition in the stems of *Obelia* (66 δ) and a deflection of the outgrowth toward the anode when the stems are placed at right angles to the path of the current (6–13.3 δ). In *Tubularia* a cathodal inhibition can be obtained for a narrow range of current strengths (5–8 δ), but when the current is increased reconstitution is inhibited at the anode. At 62.8 δ, which is about the current used by Lund, anodal inhibition occurs in *Tubularia*. In most of the current strengths used the hydranths bend toward the cathode when placed at right angles to the current.

The hydranths which emerge at the cathode in cathodal inhibiting currents are normal in structure; the inhibition is expressed by number of hydranths emerged and the time for development. Those which develop at the anode in current strengths causing anodal inhibition are small and often deformed or lacking in parts. The anodal inhibition is thus different from cathodal inhibition.

The current over a certain range (5–8 δ) is selective in its action, preventing those cathodal stems which are in a poor physiological condition from reconstituting, while the more vigorous stems are merely retarded in development. The anodal stems, on the other hand, show a higher percentage of hydranth formation over control, and thus some stems which normally would not have reconstituted hydranth are able to do so. This favorable effect at the anode may be due to cathodal inhibition as inhibition of one end of *Tubularia* in general favors reconstitution at the opposite end. It is possible that anodal stimulation also occurs.

Under certain conditions the formation of a hydranth is suppressed at the distal end and one appears at the proximal end, thus "reversing the polarity" of the stem. The stems used exhibited a strong polarity, and this phenomenon was not common. "Reversed polarity" was more frequent in the proximal halves of stems as compared with distal halves, showing that the polarity of the proximal

halves was weaker. The proximal halves take longer for regeneration as compared with distal halves.

The effects of the electric current on the regeneration of *Eudendrium* and *Pennaria* are different. In many of the experiments both forms were treated in the same dish with the striking result that *Pennaria* regenerated at the cathode and *Eudendrium* at the anode. Further, the reactions of the adult hydranth were in opposite directions. The hydranth of *Eudendrium* contracted at the cathode while that of *Pennaria* contracted at the anode. A similar effect is observed upon the contraction of the tentacles of the hydranth. In *Eudendrium* the tentacles contract on the cathodal side, and in *Pennaria* they contract on the anodal side.

This contraction of the tissues at the cathode in the case of *Eudendrium* and at the anode in the case of *Pennaria* may be a factor in the inhibition of regeneration. In normal regeneration the coenosarc migrates or grows out of the perisarc, and a hydranth forms from the outgrowth. If this cell migration or growth be prevented by a prolonged contraction of the coenosarc, no outgrowth occurs and a hydranth cannot form. Hydranths do not differentiate from the coenosarc within the old perisarc. Only when the tissue extends out of the old perisarc does the differentiation of the hydranth occur. The tissue at the inhibited end of *Pennaria* often does contract for several millimeters within the perisarc, and if the current be reversed these contracted ends do not regenerate.

However, the action of the current is something more than its effect upon contraction, since, if the current be reversed after hydranths have been regenerated by uninhibited ends, the hydranths will drop off. Merely contraction would not cause this. There is some injurious action which causes the hydranths to drop off as they usually do under adverse conditions. This injury is not permanent, however, since reversal of the current will allow the injured region to grow out. It is this injury which causes both the contraction of the hydranths and the inhibition of regeneration.

The nature of the effect of the current.—The contrasting action of weak and strong currents has been observed by other investigators. Elfving (1882) found that plant roots bent sharply at the growing zone toward the cathode in weak currents, but upon the application

of a strong current the roots curved toward the anode. The first action was a growth phenomenon, while the anodal effect was a physical effect on the anodal side of the root. Furthermore, Hardy (1913), using the onion root tip, found that the protoplasmic granules migrated to the cathode in weak currents but went to the anode with higher currents. Thus the contrasting action of weak and strong currents is not new.

With regard to the effect of the current upon the cells only possibilities can be suggested. First we might expect the migration of the protoplasm in the current. Second, there might occur a migration of ions with a subsequent redistribution of the salts depending on the relative mobilities of the ions present. This would probably lead to changes in the pH at the ends of the stems. The difference in salt concentration is important as dilute sea water accelerates hydranth formation and concentrated sea water retards development in *Tubularia*. Third, the oxidations would probably be altered by the above changes or perhaps even directly by the electric current. The change in the normal potential difference in the stem after treatment with the current suggests that the above changes occur.

SUMMARY

TUBULARIA

1. A constant electric current of about 5–8 δ inhibits reconstitution of the hydranth in stems of *Tubularia* at the cathode.

2. If the current be increased an anodal inhibition is obtained (62.8 δ).

3. As a result of cathodal or anodal inhibition, hydranths sometimes appear at the proximal end in consequence of inhibition of the distal end by the electric current. The polarity of these stems has been "reversed" by the current.

4. In most of the current strengths used the hydranths bend so as to face the cathode.

5. The anodal hydranths in strong currents (62.8 δ) are small and often deformed. The cathodal hydranths are normal but smaller than controls.

6. The electric current alters the electrical polarity of the stem.

PENNARIA

1. Anodal inhibition was obtained with all current strengths employed. The inhibition is complete at high current strengths, no anodal growths appearing. The inhibition is reversible.

2. The hydranth of *Pennaria* bends toward the anode when placed at right angles to the current.

3. The tentacles contract on the anodal side of the hydranth when the hydranth is placed at right angles to the current.

EUDENDRIUM

1. The current inhibits regeneration at the cathode, few if any cathodal outgrowths appearing. The inhibition is reversible.

2. The hydranth of *Eudendrium* bends toward the cathode when placed at right angles to the current.

3. The tentacles contract on the cathodal side of the hydranth when the hydranth is placed at right angles to the current.

LITERATURE CITED

ELFVING, F. 1882. Über eine Wirkung des galvanischen Stromes auf wachsenden Wurzeln. Bot. Ztg., **40**:258.

INGVAR, SVEN. 1920. Reactions of cells to the electric current in tissue cultures. Proc. Soc. Exp. Biol. Med., **17**:198.

HARDY, W. B. 1913. Note on differences in electrical potential within the living cell. Jour. of Physiol., **47**:108.

LUND, E. J. 1924. Experimental control of organic polarity by the electric current. IV. The quantitative relations between current density, orientation and inhibition of regeneration. Jour. Exp. Zoöl., **39**:357.

MATHEWS, A. P. 1903. Electrical polarity in the hydroids. Amer. Jour. Physiol., **8**:294.

THE DIRECTION AND MAGNITUDE OF POTENTIAL DIFFERENCES IN CERTAIN HYDROIDS

(Four figures)

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THE relation between electrical polarity and organic polarity in the hydroids has been examined by several investigators. With respect to the direction of currents along an axis there is some disagreement among different investigators using different methods. Mathews (1903) found that the distal region of a number of hydroids was negative to proximal regions. This observation was confirmed by Hyman (1920), Child and Hyman (1926), and Hyman and Bellamy (1922), the latter investigations dealing with many different forms with the general result that the dominant regions were negative. On the other hand, Lund, in a series of papers (especially 1922, 1925), found that in *Obelia* the distal end was positive to the proximal, while the middle region was negative to both ends.

It was the purpose of these experiments to determine whether there was any relation between polarity and electrical differences in potential, and further, to determine whether these electrical differences were of physiological significance in controlling regeneration. The problem was suggested and the work sponsored by Professor C. M. Child.

MATERIALS AND METHODS

The potential differences were measured by means of the potentiometer principle, using a Leeds and Northrup galvanometer having a sensitivity of about 30,000 megohms, and a Leeds and Northrup Student Potentiometer or a Type K potentiometer. The electrodes were silver-silver chloride systems prepared after the fashion described by Gelfan (1927), and a diagram of this type is found in Figure 1. The electrode consists essentially of a platinum wire, *d*, which has been silver plated and then covered with a deposit of sil-

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ver chloride, and which is immersed in the sea water of pipette, *b*. A small glass tube, *c*, filled with agar dissolved in sea water is cemented to the end of the pipette and can be changed frequently. There is also a ground glass plug, *a*, in which is sealed a copper wire making contact with the platinum. The potential difference between two of these electrodes was fairly constant and was determined and subtracted from the observed potential difference for each reading. Often the potential difference of the electrodes remained constant within 5 microvolts for an hour at a time. At other times, when exposed to large variations in temperature, there was a steady drift which did not, however, interfere with the readings, as the potential difference of the electrodes was determined in each case by dipping

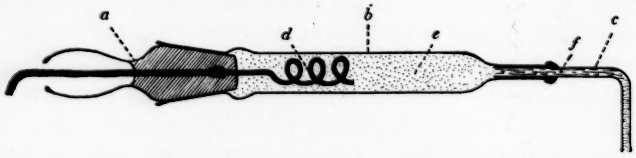

FIG. 1.—*a*, ground glass stopper; *b*, glass pipette; *c*, glass tube; *d*, silver chloride coated wire; *e*, sea water; *f*, agar dissolved in sea water.

the ends in sea water. For most of the work the sensitivity of the set-up was 5 microvolts, that is, a deflection of 1 millimeter for 5 microvolts. The sensitivity did not vary much in different experiments, as the resistance of the electrodes was greater than that of the tissues from which the potentials were led off. However, in long slender stems of *Tubularia* the sensitivity was reduced somewhat owing to the increase in resistance. In recording the potential differences the sign is always of the distal end and refers to the direction of flow of current through the galvanometer when the circuit is closed. In speaking of flow of current the older convention of flow of positive electricity is used rather than flow of electrons. For convenience the sign of the readings always refers to the condition of a distal region as compared with a proximal region, and indicates the polarity of the external circuit. Thus, for example, when the distal end is said to be positive we will expect the current to flow from the distal end through the galvanometer to the proximal end. The ab-

breviation "P.D." is often used for "potential difference." When we speak of current we mean the current which will flow when the parts in question are connected by a conductor. Actually, no currents were measured, only potential differences.

A word or two about the material is necessary since at least two different species of *Tubularia* were used for the experiments, and the reconstitution in the two forms was somewhat different. In some of the earlier work an unbranched form of *Tubularia* with a long stem was used, and, when they were cut and allowed to reconstitute, a hydranth formed at the distal end of each piece, while the proximal end, during the course of the experiment, either gave rise to a stolon or there were no signs of regeneration. It has been brought to my attention by Dr. Child that if these stems had been kept long enough a proximal hydranth might also have developed. When the stems were cut into halves the distal half reconstituted before the proximal half.

In *Tubularia crocea*, which, during the period it was used, had a short stem with many side branches, the situation was slightly different. Here almost invariably the distal end of a stem formed a hydranth and the proximal end soon after gave rise to a smaller hydranth. The same was true of the form found at Beaufort,[2] although the stems of this species were long and usually unbranched. However, during the work, this form became depressed for a period, the hydranths falling off, and the stems sometimes failing to reconstitute. When the new individuals arose from these old colonies they were shorter and had side branches. The difference in the behavior of the proximal end in the two species of *Tubularia* is probably a quantitative one, the inhibition exercised by the dominant distal region being greater in the first form described and less in *Tubularia crocea*.

Some experiments were also carried out with *Tubularia mesembryanthmum* at Naples. This is a short-branched form similar to *Tubularia crocea*. Hydranths may reconstitute at both ends of a cut stem. In addition the potentials in *Pennaria* and *Eudendrium* were measured after cutting the stems and during regeneration.

[2] I am indebted to the U.S. Bureau of Fisheries and Biological Station at Beaufort for the use of laboratory space.

DESCRIPTION OF EXPERIMENTS AND RESULTS

I. Potential Differences in *Tubularia*

This section deals chiefly with the potentials in *Tubularia*. As shown in Tables I, II, and III, however, *Pennaria* was used in some preliminary experiments to compare different methods of measuring the potentials of stems. Therefore, these data have been included with the preliminary experiments on *Tubularia*, rather than in the section dealing with potentials in *Eudendrium* and *Pennaria* during regeneration.

The most difficult problem by far, and one which has not yet been satisfactorily solved, was to establish a suitable connection between the preparations and the electrodes, and, since in many instances not only the magnitude but even the direction of the P.D. depended on the kind of contact, all of the experiments will be described. In the tables there has been no selection of data whatever, all of the results being recorded. The experiments fall into the following groups: A—sea water; B—P.D.'s on moist filter paper; C—P.D.'s in individuals supported on glass rods while the electrodes were applied; and D—those in which the preparations were placed in a moist chamber with the levels, between which the P.D. was to be measured, immersed in separate sea-water cups with the electrodes dipping into these cups.

A word about the tables may save confusion. The sign of the reading refers to the distal region of a stem as compared with a proximal region and indicates the direction of the positive current in the external circuit through the galvanometer. A negative sign then means that a current will flow from a proximal level of the stem through the galvanometer to a distal level. All readings are in microvolts. The electrode leads are described either in the tables or in the text. In all cases unbranched stems of *Tubularia* were used for reconstitution experiments, and the hydranth, together with a few millimeters of the stem, was removed. Each stem was observed individually, and the course of its reconstitution noted. The reconstituting end first becomes pink in color and the primordia of the basal and oral tentacles can be seen. When the hydranth is fully formed it is pushed out of the perisarc and this is termed "emergence." The time between cutting and emergence was taken as the time for reconstitution.

A. POTENTIAL DIFFERENCES IN SEA WATER

Since we were interested in determining the normal P.D.'s it seemed obvious that the best way of taking the leads would be in sea water. Accordingly, the preparation was placed in a flat-bottomed dish filled with sea water and the electrodes brought in contact with the various parts of both *Pennaria* and *Tubularia*. However, using the full sensitivity of the galvanometer, which for the particular experiment was 1 microvolt for 1 mm. deflection, no P.D. was observed between any of the parts of these two hydroids. This was taken to mean that either there was normally no current flowing or that almost all the current flowed through the sea water and the fraction which went through the galvanometer was not sufficient to cause a deflection.

B. POTENTIAL DIFFERENCES ON MOIST FILTER PAPER

In these experiments the hydroids were placed on filter paper moistened with sea water and the electrodes brought in contact with the hydroids by means of vertical adjustments and the P.D. of the system balanced by means of the potentiometer. The electrodes were then brought in contact with the moist filter paper and this P.D. measured and subtracted from the above value to give the P.D. of the hydroid. Most of these observations were made on *Pennaria* at Woods Hole.

Potential differences between hydranth and basal perisarc of Pennaria.—With one electrode stationary on the main stem at the base of the colony and the other electrode in contact with various hydranths, the potentials in Table I were obtained. In this particular experiment three different colonies were used, as indicated in the table. In addition, many qualitative measurements were made with the result that the hydranths were always negative to the perisarc of the stem. See also Table II, Column 2, "resting" P.D.'s.

While this method yielded consistent results in that the hydranth was negative, the magnitude of the P.D. varied with at least three external factors: (1) the pressure of the contact between the electrode and the hydranth; (2) the time of measurement after the preparation was made; and (3) the degree to which the filter paper was wetted.

In the results recorded above the P.D. was taken when the electrode was just in contact with the hydranth, so that little or no pressure was exerted. When the electrode was pressed against the hydranth the P.D. reversed and was considerably more than when measured as above. Thus in No. 9 of Table I, while the P.D. was

TABLE I

POTENTIAL DIFFERENCES IN *Pennaria*

Three colonies used: 1–5; 6–9; 10. One electrode on the hydranth and the other on the main stem at the base of the colony. Preparation on moist filter paper.

Trials	Potential Difference	Trials	Potential Difference
1	−36	6	−33
2	−22	7	−13
3	−46	8	−24
4	−25	9	−30
5	−29	10	−46

TABLE II

POTENTIAL DIFFERENCES IN *Pennaria*

A comparison of "resting" potentials and "injury" potentials due both to mechanical injury and to drying. Hydranths all of one colony. One electrode at the base of the colony and the other on the hydranth.

Number of Hydranth	"Resting" P.D.'s	"Resting" P.D.'s after 1½ Hours	"Injury" P.D.'s	Size of Hydranth
1	−17	+	+	large
2	−47	+	+	medium
3	−20	+	+	large
4	−10	− 3	+	small
5	−15	−150	+84	medium
6	−18	− 3	+30	small
7	−13	0	+20	small
8	−13	0	+15	very small
9	− 8	− 8	+45	very small
10	−15	0	+12	small
11	−15	− 31	+	small
12	−10	− 13	+	medium
13	−18	−	+	medium
14	−50	−	+	large
15	−25	−	+	medium

−30 microvolts when undisturbed, upon the application of pressure it changed to +165 microvolts. This latter current was termed an "injury" current, as it appeared when the hydranth was injured mechanically with the electrodes and also after allowing the preparation to partially dry. Table II also gives values from a preparation on moist filter paper. In this table the second column records the P.D.'s of the various hydranths just after placing the colony on moist filter paper; the third column, the potentials after 1½ hours; and the

TABLE III

POTENTIAL DIFFERENCES IN *Pennaria*

Hydranth and part of stem raised off filter paper. One electrode on the hydranth and the other on the stem.

No.	P.D.	No.	P.D.
1	−245	18	−320
2	−230	19	−230
3	−340	20	−285
4	−170	21	−780
5	−150	22	−380
6	−245	23	−110
7	−187	24	− 90
8	−126	25	−100
9	−330	26	−130
10	−330	27	−100
11	−200	28	−100
12	−380	29	−200
13	−263	30	− 80
14	−248	31	− 76
15	−283	32	− 65
16	−153	33	−105
17	−273	34	−210

fourth column, the results upon mechanically injuring the hydranth with the electrode.

The "resting" P.D.'s are all negative; after an hour and a half they either become positive or less negative with one or two exceptions; and with injury they all become positive. The "injury" currents in this experiment are not as great as those obtained when a fresh preparation is injured. A rough estimate of the size of the hydranths is included in the table, as in general it was noticed that the larger hydranths gave higher "resting" P.D.'s.

While measuring the P.D.'s as described above, it was noticed that when the hydranth was lifted off the filter paper the potential

rose to about ten times its original value. Thus, for example, one preparation measured on moist filter paper gave a difference of potential of −47 microvolts between hydranth and pressure, while when the hydranth was raised from the paper the P.D. rose to −365 microvolts. If the preparation was lowered so that the hydranth came in contact with the moist filter paper, the P.D. dropped to its original value. A number of measurements were taken with the main stem of a colony of *Pennaria* on moist filter paper and the branches in air, and these results appear in Table III.

C. POTENTIAL DIFFERENCES IN *Tubularia* IN AIR

About this time some *Tubularia* were obtained, and the experiments were continued with this form. The *Tubularia* seemed in good condition and was a straight unbranched form with individuals ranging from 2 to 7 cm. in length. In this form the stems when cut produced a hydranth at the distal end, while at the proximal end either a stolon formed or else no reconstitution took place. Tables V–XVI record data on this form. Unless otherwise stated, the stems were measured by placing them on glass supports over a dish of sea water and the electrodes were brought into contact with the hydroid by means of vertical adjustments. The electrodes were then immersed in the sea water of the dish to determine their P.D., and this value was subtracted from the P.D. of the preparation. In this way the entire length of the stem was in air and the current was not short-circuited, as in the case of the moist filter paper preparations.

1. *Potential differences in full-grown and in reconstituted pieces of Tubularia.*—Using this method the potentials of full-grown hydroids were determined by cutting the individuals at the base and placing one electrode on the hydranth and the other on the uninjured perisarc about 30 mm. from the hydranth. The cut pieces were about 35–40 mm. long (Table IV). The P.D.'s of stems which had recently reconstituted a hydranth are shown in Table V. These latter pieces had been used as a control for another experiment, and all of them had reconstituted a hydranth at the distal end and had formed a stolon at the proximal end. The measurements were taken about 48 hours after the hydranths had emerged, and both hydranths and the stolons had grown out so that a region of about 3–6 mm. at each end

was not covered with the thick perisarc of the rest of the stem. The electrodes were placed on these regions, and thus the potentials are between two growing regions and not between the perisarc and the hydranth, as in previous experiments. In both tables with but one

TABLE IV

POTENTIAL DIFFERENCES IN *Tubularia*

P.D.'s of pieces about 35–40 mm. long. One electrode on hydranth, the other on the perisarc of uninjured stem. Distance between electrodes about 30 mm.

No.	P.D.	No.	P.D.
1	−720	8	−510
2	−480	9	−650
3	−350	10	−200
4	−350	11	−300
5	−400	12	−685
6	−340	13	−140
7	−430		

TABLE V

POTENTIALS IN *Tubularia*

P.D.'s of pieces about 25–30 mm. long, which had reconstituted a hydranth at one end and a stolon at the other. Electrodes brought in contact with hydranth and stolon.

No.	P.D.	No.	P.D.
1	+ 210*	6	−1585
2	− 340	7	− 235
3	−1260	8	−1350
4	− 350	9	− 400
5	− 730	10	− 975

* The hydranth on this individual disintegrated 12 hours later.

exception the hydranth is negative and it may be significant that in this one the hydranth disintegrated about 12 hours after the reading was taken.

2. *Potentials during reconstitution.*—In these experiments pieces of *Tubularia* were kept in running sea water and the potential difference between the distal and proximal ends taken from time to time by placing the stems on glass supports and bringing the electrodes into contact with the ends as previously described. In a few days a

hydranth usually formed at the distal end of the cut stem. In some cases a smaller hydranth appeared at the proximal end and sometimes, for one reason or another, the proximal hydranth appeared

TABLE VI

POTENTIALS IN RECONSTITUTING PIECES OF *Tubularia*

Individuals old with many medusae. Electrodes applied to the distal and proximal cut ends.

No.	Length in mm.	P.D. after Cutting	12½ Hours Later	Order of Reconstitution
1	40	−320	−170	5
2	40	−130	−325	6
3	30	−520	−250	8
4	30	−710	−250	4
5	45	−400	lost	.....
6	30	−560	−300	3
7	35	−340	−890*	1
8	65	−400	−365	2
9	30	−440	−170	7
10	50	−270	−190	9

* Hydranth well developed.

TABLE VII

POTENTIALS IN *Tubularia*

P.D.'s of cut pieces showing fluctuations after cutting. Electrodes in contact with cut ends.

No.	P.D.'s during First 10 Min.	After 1 Hour
1	+300, remaining so	+ 70
2	First +, then −, then +270	+350
3	First −, then +10	+340
4	First −, then +170	−510
5	First +, then −70	+835
6	First +, remains +420	+180
7	First −, then +760	+370
8	First −, then +160	+330
9	First −, remaining −220	−210
10	First −, then +120	+200

first. The entire length of the stem was used in some experiments, other stems were cut into halves, and still others into thirds. Tables VI–VIII give data for the entire stem; IX–X for halves; and XI and XII for thirds.

Immediately after cutting, the distal end of a stem is negative,

and 12 hours later, after reconstitution has begun, it is again negative (Table VI). However, a few minutes after cutting, the distal end becomes positive and remains so for about 1 to 2 hours (Tables VII and VIII A). The P.D.'s, after cutting, may be variable and

TABLE VIII

POTENTIAL DIFFERENCES IN *Tubularia*

Stems cut off at the base and the hydranth removed with a few mm. of stem. Electrodes in contact with cut ends.

	Hours after Cutting	No. of Pieces				
		1	2	3	4	5
Exp. A: Length 25 mm.	0*	−213	+240	0	0	.....
	1½	+ 80	+125	+130	+370	+230
	5	+ 75	−125	−130	−200	−440
	9	.....	−240	− 75	−270	−320
	21	−185	−240	+270	−125†	−725†
	31	−140†	−230†	−260‡	−255†	−455‡
Exp. B: Length 20 mm.	0	−250	−200	−330	−410	−225
	3	−540	−140	−450	−485	−160
	17½	−250	−210	−760†	−610†	−350
	28	First + then −70	First + then −415	First + then −	Varies ca. −100	First + then 0
	Hydranth emerges	64 hrs.	64 hrs.	40 hrs.	40 hrs.	.....
Exp. C: Length 50–55 mm.	0	−100	−280	−240	−200	−270
	3½	−310	+330§	−450	−120	−400§
	18½	−260	+140§	+100	+150	+150§
	28½	.....	.....	+600	.....	.....
	60	Hydranths emerged at proximal end of No. 3 and 4.				

* Potentials variable immediately after cutting.

† Distal hydranth developing.

‡ Distal hydranth emerged.

§ Coenosarc bright red at distal end and contracted within perisarc; no reconstitution. No reconstitution in No. 1.

sometimes become positive while being measured (Table VII). However, they become negative later and remain so (Table VIII A, B, and C).

It is interesting to note in Table VIII C that the distal ends of Nos. 3 and 4 are positive about 18 hours after cutting, and a hydranth forms at the proximal end instead of the distal end. The others did not reconstitute.

Since there is a marked difference in the rate of reconstitution of distal and proximal halves it might be expected that there will also be differences in P.D. Tables IX and X show that immediately after cutting the potentials in the distal halves are much greater than

TABLE IX

POTENTIAL DIFFERENCES IN *Tubularia*

Individuals old with many medusae. Stems cut into distal and proximal halves. *D*, distal half; *P*, proximal half.

No.	Length in mm.	Hours after Cutting		Approximate Order of Reconstitution
		0	12	
1*D*	20	−920	−255	3
1*P*	20	+ 80	−480	1
2*D*	25	−410	−110	5
2*P*	25	0	−230	8
3*D*	30	−215	−620	2
3*P*	30	+ 70	− 40	6
4*D*	20	−630	−280	9
4*P*	20	0	+220	7
5*D*	30	−265	−100	4
5*P*	30	0	− 40	10
6*D*	20	−295	.....	.....
6*P*	20	−205	.....	.....
7*D*	30	−950	.....	.....
7*P*	30	− 20	.....	.....
8*D*	25	−415	.....	.....
8*P*	25	− 60	.....	.....
9*D*	20	−370	.....	.....
9*P*	20	− 20	.....	.....
10*D*	20	−385	.....	.....
10*P*	20	− 25	.....	.....

those in the proximal ones. As Table IX records, the proximal half may be positive or show no P.D. Later these differences may not appear, due, probably, to the fact that some of the proximal pieces may reconstitute a hydranth before the distals. The distal end of proximal halves for some time after cutting is weakly negative or even positive (Table X).

When the stems are first cut there is a more or less uniform difference in the magnitude of the P.D.'s of proximal, middle, and distal thirds of *Tubularia*. Thus, in general, the distal end of the distal third is negative, that of the middle third is either less negative or is positive, and the distal end of the proximal third is usually positive (Tables XI and XII). These differences correspond to differences in

TABLE X

POTENTIALS IN RECONSTITUTING PIECES OF *Tubularia*

Individuals about 40 mm. long, cut into proximal and distal halves. *D*, distal half; *P*, proximal half. Temperature: 19° C.

No.	Hours after Cutting			
	0	3	17½	28
1*D*	−330	−275	−680	−310
1*P*	−130	+100	+620	−390
2*D*	−190	−160	−330	−335
2*P*	−110	−110	0	−375
3*D*	−100	−275	−330*	−155
3*P*	−100	+150	−175	− 45
4*D*	− 55	−180	−240*	−235
4*P*	− 25	+330	0	−220
5*D*	−110	− 80	−240*	−200
5*P*	−100	+110	−140	−450

* Distal hydranth developing.
All pieces develop a hydranth.

the rate of reconstitution, the distals forming hydranths first, the middle pieces second, and the proximals last.

The order in which the stems were measured after cutting is noted in Table XI as it was thought that, since the P.D. usually becomes positive a short time after cutting, the differences in the distal, middle, and proximal pieces might be due to the fact that the distal piece was measured first, the middle second, and the proximal third. However, as Table XI shows, such is not the case, and the differences occur regardless of the order in which the pieces are measured.

Table XII gives more complete data on the P.D.'s of thirds, and shows the typical condition after cutting. After an hour all the

pieces save two have positive distal ends, and at 6 hours they all become negative except one. It will be remembered that the entire stem goes through this positive stage after cutting. After 6 hours the potentials are variable and little can be said about the relation between P.D. and reconstitution.

TABLE XI

POTENTIALS IN *Tubularia*

Length 45–65 mm. Individuals old with many medusae. Cut into thirds. *D*, distal; *M*, middle; *P*, proximal. Temperature: 19° C.

No.	Length in mm.	Hours after Cutting			Order of Emergence of Hydranths
		0	11	111	
1*D*	22	+110[1]	−340	−390[a]	1
1*M*	22	− 25[2]	−210	−270	8
1*P*	22	+ 35[3]	−175	−150	10
2*D*	22	−330[4]	−380	−320[d–c]	.....
2*M*	22	+120[5]	.....	+245	5
2*P*	22	+140[6]	− 10	+ 90	.....
3*D*	20	−370[9]	−430	−130[b]	2
3*M*	20	+ 20[8]	0	− 60	3
3*P*	20	+105[7]	+ 5	+ 50	.....
4*D*	22	−210[12]	.....	−345[c–d]	7
4*M*	22	− 55[11]	.....	−135	6
4*P*	22	+ 45[10]	.....	−280	4
5*D*	15	−210[13]	.....	− 70[e]	.....
5*M*	15	−160[14]	.....	−230	9
5*P*	15	− 40[15]	.....	.....	.....

[1], [2], [3], etc. = order of measurement.
a, *b*, *c*, etc. = order of reconstitution of distal thirds as judged by condition of hydranth within perisarc.

If we were to come to any conclusion from the results of these experiments using the technique of previous workers we would say that the distal end of *Tubularia*, when dominant, is negative externally and that in long stems there is a second negative region at the proximal end. Certainly, using all the evidence from the work on *Pennaria* and *Tubularia*, we could hardly fail to draw the general conclusion that in these hydroids the dominant region is negative. This is in accord with most of the previous investigations except that of Lund (1925), who worked on *Obelia*.

While the general results of the foregoing experiments are unquestionable, there are, however, individual cases which seem to contradict the idea that the dominant region is negative. Thus, in Table VIII A, No. 3, at 21 hours, the P.D. of a developing hydranth is +270 microvolts, while that of No. 5, which is developing parallel to it and which emerges at the same time, is −725 microvolts. In

TABLE XII

POTENTIAL DIFFERENCES IN THIRDS OF *Tubularia*

Individuals about 50–60 mm. long, cut into thirds. *D*, distal third; *M*, middle third; *P*, proximal third.

No.	Hours after Cutting									Time for Emergence (Hours)
	0	1	6	10	22	27	34½	48½	71	
1*D*	−170	+250	−200	− 70	+285	−190	− 50	+210	−620†	53
1*M*	−330	−190	−560	− 70	−100	− 80	− 75	−190	−400*	93
1*P*	+205	+150	+ 90	+250	−840	−260	+130	−310	+335*	83
2*D*	−210	+225	−210	+250	+295	+170	− 60	−105	−710	115
2*M*	−100	+130	−460	−160	− 90	−260	− 55	+ 50	−300*	93
2*P*	+135	+120	−220	− 5	+360	+100	−350	+220	+285	.....
3*D*	−175	+390	−630	+ 75	−540	−250	−350	−150	− 90*	79
3*M*	−220	+300	−240	+ 90	− 40	−130	+160	−110	−260*	77
3*P*	+155	+220	−250	−320	+580	− 80	+495	−110	−290*	81
4*D*	− 90	+ 50	− 60	+ 70	− 80	+ 85	+ 65	−100	+ 30*	77
4*M*	− 95	+515	−225	+110	+190	+120	+220	+ 70	+210*	93
4*P*	− 75	0	−470	−210	− 45	−295	− 70	−280	.....	.....

* Distal hydranth developing.
† Distal hydranth emerged.

Table VIII B, Nos. 1, 2, and 3, at 28 hours, are first positive and then negative, remaining negative. The negative value was selected, as the transitory positive P.D. was thought to be due to handling and manipulation of the electrodes against the ends. In Table IX No. 4P, 12 hours after cutting, has a P.D. of +220 microvolts, while all the rest are negative; yet it reconstitutes a hydranth. Tables X and XI also show exceptions which apparently develop as well as those which follow the rule.

These and other experiments showed that the method of measuring was not entirely satisfactory, for the P.D. often varied while

measuring and even reversed, and once a contact was established with the ends of a piece, the least movement of the electrodes changed the P.D. Then, too, the P.D.'s could not be followed for a long period as the preparations would dry and the perisarc collapse.

D. POTENTIAL DIFFERENCES IN *Tubularia* IN MOIST CHAMBERS

In order to overcome some of these difficulties a moist chamber was devised in which reconstituting stems would live and develop. Several different types were tested and the one in Figure 2 finally used. It consists simply of a block of paraffin with two cups hollowed out and filled with sea water, into which the ends of the preparation

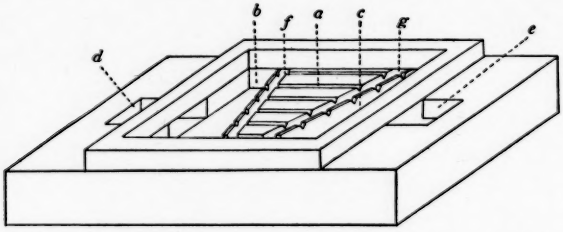

FIG. 2.—Moist chamber for the measurement of potential differences in *Tubularia*. *a*, depressions in which the stems of *Tubularia* were placed; *b*, *c*, cups filled with sea water so as to make a liquid junction with the ends of stems; *d*, *e*, cups in which the electrodes were immersed; *f*, *g*, grooves in paraffin.

are immersed. The triangular shape of the area between the cups allows for a stem of any length, and also for immersion of a piece to any level by merely changing its position. The stem is placed in one of the grooves, *a*, with the ends dipping into the sea water of cups *b* and *c*, which, in turn, connect with cups *d* and *e* on the outside of the chamber. The grooves *f* and *g* were cut into the paraffin to prevent water from creeping along the stem and changing the point of contact of the sea water and the stem. The whole chamber is covered with a glass plate and measurements are made by dipping the electrodes in cups *d* and *e*. This arrangement not only does away with handling the preparation but also insures a moist atmosphere and maintains the same contact throughout reconstitution. Thus, the P.D.'s can be taken at intervals or continuously for any length of time. Water can be run through the cups continuously, but this was

not done in most of the experiments since it was found that little difference in the P.D.'s resulted.

In order to test out the moist chamber as one giving representative values in *Tubularia*, the following experiment was carried out: Two individuals, which were nearly alike in appearance, were selected, the side branches and a part of the base removed, and the pieces termed "Preparation A" and "Preparation B" respectively. Preparation A was immediately placed in the moist chamber with the hydranth in one cup and the proximal end in the other, while B

TABLE XIII

POTENTIALS IN *Tubularia*

Moist chamber in Figure 2 used. P.D.'s taken between the hydranth and the proximal cut end. *A* and *B* about 25 mm. long. Measurements begin after cutting.

Prep.	Minutes after Cutting								
A	0	10	30	31	45	165	195	210	240
	−170	−150	+40	*A* returned to sea water				+210	+95
B	*B* kept in sea water			+50	+	+390	+380	*B* returned to sea water	

was returned to sea water. The P.D. of A was taken for 30 minutes and it was then returned to sea water and B was placed in the same chamber and its P.D. taken at intervals. The preparations were again exchanged to see if the moist chamber was a suitable place in which to keep *Tubularia*. Table XIII shows that there is little difference in the P.D.'s of the two preparations, comparing the periods during which one was in sea water and the other in the moist chamber. Thus A, which was kept in the moist chamber for 30 minutes, gave a P.D. of +40 millivolts, while B, which was kept in running sea water for the same period, gave +50 millivolts. It should be remarked here that *Tubularia crocea*, a shorter, much branched form, was used in this experiment. Stems of this form almost invariably reconstitute a hydranth at the distal end first and later a smaller hydranth at the proximal end. In the former experiments usually the

distal hydranth developed, the proximal one not appearing during the course of the experiment.

1. *Potential differences in whole stems during reconstitution.*—For these experiments several moist chambers of the type already described were made and cut stems placed in them, covered with glass plates, and allowed to reconstitute without further handling. With this procedure we have a physiological axis with a dominant region usually at the distal end, the P.D. of which can be determined con-

TABLE XIV

POTENTIAL DIFFERENCES IN *Tubularia*

Individuals young. Stems about 20 mm. long.

No.	Hours after Cutting					
	0	1½	8	17½	40½	47½
1	− 70	− 70	−450	−350	− 70†	+330
2	−220	0	−150	−550	0*	+ 40
3	+280	−300	−230	− 50	+140†	+150
4	−120	−240	+160	−350	+430†	+ 80
5	−230	− 30	−130	−600	+ 70†	+230
6	−950	−270	+350	−200	+170†	+240
7	+100	+390	−140	+800*	+270†	−140
8	−270	−300	−140	+100*	−160†	+290
9	−900	−200	−180	−350	+550*	+200
10	−390	−320	−230	−900	+ 50†	− 80

* Distal hydranth developing.
† Distal hydranth emerged.

tinuously or from time to time without the necessity of manipulation or the establishing of new contacts.

With this chamber the potential differences in short, rapidly reconstituting stems are much more constant, as shown by Table XIV. These stems were young, unbranched stems which were obtained at Beaufort, North Carolina. The distal end in all ten pieces is almost uniformly negative for 17½ hours after cutting, with the exception of Nos. 7 and 8, where a hydranth is developing and the P.D. is positive. At 40½ hours the P.D.'s are all positive except Nos. 1, 2, and 8.

Tables XV and XVI give data on old stems of *Tubularia*, and here the times required for reconstitution are more variable and the

TABLE XV

POTENTIAL DIFFERENCES IN *Tubularia*

Individuals old. Stems about 35–40 mm. long.

HOURS AFTER CUTTING	NO. OF PIECES				
	1	2	3	4	5
0	+120	− 100	+ 120	+580	−300
3	+210	+ 470	− 150	+280	− 40
6	− 80	− 730	+ 130	+160	−160
11	− 80	−1010	− 60	+350	−710
23	+200	− 270	+ 220*	+430*	−470
28	+ 25	+ 20	+ 650	+700	+180
31½	− 40	+ 30	+ 240	+820	+520
37½	+450	− 60	− 500‡	+970†	+670*
47½	+400*	− 200	+ 850	+770	+100§
55½	+540	− 50	+1150	+200	− 80
72	+300†	− 310	+ 620	+250	+ 80

* Distal hydranth developing.
† Distal hydranth emerges.
‡ Distal hydranth well developed but withdrawn into perisarc.
§ Coenosarc contracted at both ends.

TABLE XVI

POTENTIAL DIFFERENCES IN *Tubularia*

Entire stems used; 1–5 old individuals; 6–10 somewhat younger individuals. First measurement about 10–15 minutes after cutting.

HOURS AFTER CUTTING	NO. OF PIECES									
	1	2	3	4	5	6	7	8	9	10
10 min.	− 90	− 85	− 200	+ 80	+ 20	+ 200	−270	+ 40	−460	+550
1	− 90	+ 55	− 220	− 70	−110	− 250	+300	− 90	+340	−190
3	−460	+130	+ 330	+ 620	+130	− 570	+230	+150	−130	−210
7	−345	− 70	+ 900	+ 940	−170	−2010	+120	+380	+130	−580
10	−240	+ 40	+ 860	+ 905	−310	−1360	−190	+110	−150	−165
22½	+350*	+290*	+1030	+1190	− 80	+1110*	−360	+390*	−140	−220
30	+300	+ 50	+1080	+ 950	+210	− 15††	+490	+770	+320	+100
38	+140	+105	+ 680	+ 125	+230	+ 230	+500	+100†	+ 80	+220
46	−170†	− 40§	+ 700‡	+ 310*	− 50**	+ 70†	+270‖	+150†	− 40	+600
54	−240	0	+1100	+ 800	+380	+ 140	+810	+230	− 40‡‡	−600‡‡
Length mm.	32	30	35	32	37	29	23	23	21	25

At 71 hours the preparations are in poor condition and the hydranths begin to disintegrate. Controls for this experiment regenerate in about 33 hours.

* Distal hydranth developing.
† Distal hydranth emerged.
‡ Hydranth developing at both ends.
§ Distal hydranth emerged, proximal one emerging.
‖ Hydranth well developed but still within perisarc.
** Distal hydranth disintegrating within perisarc.
†† Coenosarc contracted at distal end.
‡‡ No regeneration.

P.D.'s are likewise variable. No. 4 in Table XV and No. 8 in Table XVI are exceptional cases, for the P.D.'s are positive after cutting and remain so throughout the period examined. However, the distal end becomes more positive while the hydranth is developing than after cutting, so that the variation is in the same direction as in

TABLE XVII

POTENTIAL DIFFERENCES IN *Tubularia mesembryanthmum*

Measured by placing stem in moist chamber each time. Stems kept in running sea water between readings. Two colonies used, *A* and *B*. Direction and magnitude of potential as before.

Description and Length in mm.	0	15 Hours	24 Hours	37 Hours	48 Hours	Hydranths Emerged
*A*21	−400	−310	−130	−250	−230	84
*A*25	−150	−340	+250	+250	H	43
*A*17	−190	0	0	−400	H	43
*A*14	−210	−230	+ 50	+ 50	−130	.....
*A*18	−150	−120	− 50	−310	−130	60
*A*20	− 90	−210	+150	+120	− 80	.....
*A*20	−400	−280	−100	−210	+320	60
*A*15	−260	var.	+ 80	−170	− 80	.....
*A*13	−240	var.	+300	+200	− 70	.....
*A*16	−160	−230	− 60	.....	−140	.....
*B*17	+130	−160	−100	−180	− 30	.....
*B*22	+130	0	+180	+150	−140	66
*B*15	+120	− 30	+130	+120	0	84
*A*17	−170	−130	+120	−180	+400	66
*B*18	+130	−130	−120	−130	−140	60
*A*18	−110	−130	−120	−130	−200	60
*B*21	+ ?	−130	0	−130	−100	60
*B*21	+ 70	−230	+ 30	−100	−100	94
*B*25	+120	−140	+100	− 80	H	43
*A*15	−230	−100	−100	−100	−100	.....

young stems. Table XVI shows several irregularities in development giving us irregularities in the P.D.'s. Nos. 3, 4, 5, 7, 9, and 10 failed to regenerate completely.

For comparison some measurements were made at Naples by placing the stem in the moist chamber for each measurement and returning the stem to running sea water during the intervals. These data are found in Table XVII. Here an interesting difference between two colonies A and B is shown. In A the distal end is invariably negative after cutting. In B it is invariably positive. However,

both A and B stems are negative at 15 hours and both groups regenerate. The potentials at 24 and 37 hours are variable.

2. *Potential differences in distal, middle, and proximal thirds of Tubularia during reconstitution.*—In these experiments long stems were selected and cut into approximate thirds, and each piece was placed in a moist chamber for measuring the P.D.'s during development. Referring to Table XVIII the pieces when first cut indicate a gradient in the stem with the two ends similar qualitatively but not quantitatively. The potentials are greatest in the distal thirds and the direction of the positive current is from the proximal to the distal end, while in the proximal thirds the P.D.'s are less and sometimes the direction is reversed. The polarity of the middle pieces is usually the same as that of the distal third, but the P.D. is smaller. At $3\frac{1}{2}$ hours after cutting the distal ends of all except one piece are negative. At $9\frac{1}{2}$ hours most of the stems are still negative, but at $21\frac{1}{2}$ hours, with the appearance of the regenerating hydranth, the distal thirds become positive. After the hydranths emerge the distal ends are positive.

3. *Potential differences along the intact stem of Tubularia.*—It will be seen from the tables that there is considerable variability in the sign of the P.D.'s of similar stems. Part of this variability is probably due to the fact that we are dealing with two cut ends, each of which is capable of regenerating but at different rates. After cutting, the P.D. will depend on the injury and recovery of the two ends, and this probably accounts for the fluctuations recorded for the first few minutes after cutting.

Likewise, during regeneration, we have similar processes going on at the two ends, and the P.D. will be expected to fluctuate with fluctuations in the relative rapidity of these processes. Thus, at one time the distal end may be negative while later the proximal end becomes negative for some time, due to an increase in the rate of whatever processes contribute to the P.D. The distal end may return to the negative condition when its activity exceeds that of the proximal end. No. 2 D, Table XVIII, shows such a series of changes (compare $21\frac{1}{2}$, $27\frac{1}{2}$, $33\frac{1}{2}$, and $45\frac{1}{2}$ hours).

In order to avoid dealing with two variable regenerating regions some experiments were carried out in which one of the ends made

contact with the electrode while the other lead was taken from an uncut resting surface along the stem. This was done in two ways. In one set of experiments the stems were placed in a chamber with about half of the stem immersed in one sea-water cup and one end of

TABLE XVIII

POTENTIAL DIFFERENCES IN *Tubularia*

Stems cut into thirds. *D*, distal third; *M*, middle third; *P*, proximal third. Each third about 14–18 mm. long.

No.	Hours after Cutting						
	0	3½	9½	21½	27½	33½	45½
1*D*	−550	+130	− 60	−240*	+260	+ 40†	−160
1*M*	−170	−220	− 55	+440	+420*	+ 20	+ 80‡
1*P*	+ 50	−320	−600	−340	− 10	−250*	− 70†
2*D*	− 40	−165	−100	+330*	+130	−190	+130†
2*M*	−280	−160	− 25	− 80	+ 85	−100*	+ 50†
2*P*	−190	−140	− 80	−270	− 60	−140*	+ 50†
3*D*	−100	− 90	−250	+220*	.....	+ 40	+ 30
3*M*	− 60	−280	− 40	+ 60	− 60	− 50	+110
3*P*	+160	−300	−180	−430	−280	−230	−140*
4*D*	−150	−240	−130	+210	+140	+150*	+140†
4*M*	+ 40	0	+ 30	−130	+ 30	+ 70*	+ 80†
4*P*	+ 70	−110	− 10	+110	+100	0*	+150†
5*D*	−200	−140	0	+140*	−150	− 90	+ 10
5*M*	−210	−100	− 50	− 80	− 50	− 50*	+ 40
5*P*	+ 50	− 50	− 25	− 60	−130	− 90*	+110

* Distal hydranth developing.
† Distal hydranth emerged.
‡ Distal hydranth and proximal hydranth emerged.

the stem in the other sea-water cup. The other method involved the use of a chamber with three sea-water leads.

With the first method the same type of moist chamber was used as shown in Figure 2, but instead of immersing both ends of the stem, only one end was in contact with sea water and the entire half or third of the other end was completely covered with sea water. In this way the potential differences between the middle and the ends were determined by means of three series of preparations. In the first series, A (Table XIX), the distal end was dipping into one sea-

water cup and the proximal region was completely immersed up to the middle of the stem. This presumably short-circuits the P.D. along the proximal half so that the P.D. between the middle and the distal end is obtained. Likewise, by immersing the whole distal half in one cup and the proximal end in the other, it is possible to deter-

TABLE XIX

POTENTIAL DIFFERENCES IN *Tubularia*

A, potentials between the distal end and the middle of the stem; *B*, potentials between the middle and the proximal end; *C*, potentials between the distal end and the proximal end. Sign indicates the direction of flow through the galvanometer and refers to the distal regions. See text.

Preparations		Hours after Cutting							
		0	2	6	9	21	27	35	45
A	1	−780	−600	−130	−120	−170†	+140	− 50‡	.....
	2	−300	−640	−190	−220	− 60†	− 80‡	− 50	.....
	3	−750	−360	− 90	−140	−100†	− 40‡	− 50	.....
	4	−600	−380	−230	−150	−190†	− 50‡	− 60	.....
	5	−950	−190	−200	−140	−160†	− 50‡	−100	.....
B	1	− 60	−140	0	+ 80	+ 60‡	+130	+120	.....
	2	+ 20	0	+ 80	+ 70*	.....	.....	.....	.....
	3	+260	+110	+ 80	+120	+ 90†	+ 20‡	+ 20	.....
	4	+180	− 70	− 30	+ 50	+ 50‡	0	+ 10	.....
	5	−200	0	+160	+ 80	+120†	+ 30‡	0	.....
C	1	−250	+ 50	− 30	0	+740†	+240	+ 80	.....
	2	−540	−260	−190	−310	+280†	+380	+ 40‡	.....
	3	0	+ 60	+ 60	− 10	+ 60†	+ 40	+100	.....
	4	−+	− 60	−170	−340	−250†	− 50	−160	+100‡
	5	−+	−160	0	+110	+ 70†	+240	+160‡	−?‡

* Preparation discarded.
† Distal hydranth developing.
‡ Distal hydranth emerged.
−+ Potentials variable.

mine the polarity of the proximal half (B of Table XIX). A third set of preparations (C) was made with the ends only making contact with the sea water. With regard to the polarity in Table XIX, in A the sign refers to the distal end, in B to the middle, and in C again to the distal end.

The data then tell us that almost throughout reconstitution the currents flow from the middle region to the two ends in the external

circuit; in other words the middle region is positive to the two ends. As already noted, the distal end is negative to the proximal end during early stages of development and in later stages becomes positive.

In the same way thirds of intact stems of *Tubularia* were measured with the results given in Table XX. The currents flow from the un-

TABLE XX

POTENTIAL DIFFERENCES IN *Tubularia*

Stems about 60 mm. long. Stems immersed to different levels so that the distal third, the middle third, the proximal third or the entire stem could be measured. See text. Negative sign indicates that the current will flow from a proximal level through the galvanometer to a distal level. *D*, distal third; *M*, middle third; *P*, proximal third; E, entire stem.

No.	Hours after Cutting											
	0	1½	3½	5½	7½	9½	13½	22½	25½	29½	33½	36½
*D*1	− 40	0	− 60	− 30	− 45	− 75	−140	− 75	−115*	− 20	+160	+140†
*D*2	− 80	−100	− 90	− 40	− 95	−220	−120	+100	− 20*	+170	+190†	− 20
*D*3	− 60	−120	− 40	−115	−165	−225	−175	+ 60	+ 50*	− 90*	+ 20	− 60†
*D*4	− 90	− 50	−110	−110	−285	−230	−250	− 30	− 40*	+120	+ 20†	+ 30
*M*1	− 90	− 30	− 70	− 35	− 45	− 45	0	− 60	− 30*	− 30†	0	0
*M*2	−100	30	− 10	0	0	− 15	− 20	− 30	− 20*	+ 10†	+ 15	+ 20
*M*3	+130	0	+ 60	− 70	− 40	+ 40	+110	+130	+ 10*	− 35	− 10†	− 20
*M*4	−220	− 70	−190	−110	−110	− 80	− 40	− 80	− 50*	− 60	− 25†	− 30
*P*1	− 60	0	0	+ 30	+130	+ 75	+ 60	+ 40	+ 80*	+ 50	0	+ 20†
*P*2	+ 40	+ 50	+ 70	+ 95	+ 90	+ 65	+ 80	+130	+ 40†	+ 15	+ 20	+ 25
*P*3	−130	+130	+ 40	+115	+160	+195	+190	+130	+ 0‡	+ 15§	+ 30	+ 40
*P*4	− 60	+180	+180	+210	+220	+230	+160	+230	+150†	+ 30	+ 70	+110
*E*1	−160	−100	− 60	−755	−150	−235	−220	− 20	+ 70*	+ 60	0	− 80
*E*2	− 60	+270	+160	+100	+230	−410	+280	−265	+320*	+450	+400	+ 50
*E*3	− 30	−150	−100	− 80	+ 20	− 25	− 20	+410	+540*	+410	+140	+280†
*E*4	−100	+ 60	+ 90	− 70	−110	−100	−195	+150	+ 60*	+ 80	+120†	− 70

* Distal hydranth developing.
† Distal hydranth emerged.
‡ Distal hydranth emerged, proximal one developing.
§ Distal and proximal hydranths emerged.

injured regions to the cut ends, as in the case of the foregoing experiments. The potential difference in the distal third, however, is much less than that of the distal half; consequently, when the changes in the distal end take place tending to bring about a lower potential, as we witnessed above, the distal end may become positive when the

hydranth emerges (Table XX, D2 and D4 at $33\frac{1}{2}$ hours, D1 at $36\frac{1}{2}$ hours). In the proximal thirds the polarity is soon established and remains the same throughout reconstitution, the proximal end being negative. The middle thirds are interesting in that they show for the most part the distal regions negative to the proximal. Other tests showed that, in general, currents along the stem flowed from proximal to distal regions. The P.D.'s between the distal and proximal cut ends of entire stems of the same length as those used for thirds are variable and probably correspond to irregularities in reconstitution (E1–E4). Although all of the stems began to reconstitute a hydranth, only two out of four emerged. All of the hydranths emerged in the case of thirds. The entire stems were long (60 mm.) and perhaps in E1–4 too much of the stem was out of water for normal development. However, the polarity of the distal end is negative until the hydranth can be seen within the perisarc, at which time it becomes positive.

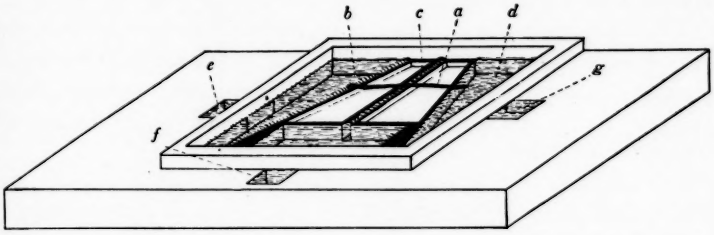

FIG. 3.—Moist chamber for the measurement of potential differences in *Tubularia*. *a*, stem of *Tubularia*; *b*, *c*, *d*, cups filled with sea water making contact with stem; *e*, *f*, *g*, cups in which the electrodes were immersed.

For the purpose of checking up on the P.D.'s obtained by the method just described, a type of moist chamber was designed having three cups instead of two, and so arranged that contact between sea water and the stem could be made at both ends and also in the middle (Fig. 3). Cups *b*, *c*, and *d* are the sea-water cups which lead into cups *e*, *f*, and *g*. The stem is placed in the chamber, a glass plate put over the preparation, and the potential differences measured by dipping the electrodes into the sea water. The shape of the cups allows for stems of different lengths.

With these preparations the results in Table XXI were obtained. In accordance with previous findings, the two ends are negative during the entire period of development but, while the distal end is negative to the proximal after cutting, it may become positive in late reconstitution. If we assume that the chemical reactions at the contact in the middle of the stem do not change appreciably during reconstitution of the ends, we may describe the changes in electrical potential in the following manner. After cutting, the distal end is usually negative to the middle, but the P.D. becomes less during the process of reconstitution. Some chemical changes take place which make the distal end less negative. At the same time the proximal end becomes more negative and both of these factors tend to make the distal end positive with respect to the proximal end. Both the distal and proximal ends, however, remain negative to the middle, and the relative strength of these two potentials will determine the P.D. between the two ends.

4. *Variations in P.D. caused by factors other than reconstitution.*—The reconstitution of sections of old individuals of *Tubularia* in a moist chamber is often irregular and injury or activation may create a P.D. Contraction of the tissue at the base of the hydranth, withdrawing it into the perisarc, causes the end to become negative. In a few cases a hydranth developed but was pulled back into the perisarc, giving these "action" currents. In one experiment this situation could be brought about in newly reconstituted stems by removing the glass plate from the moist chamber and exposing the piece to air. The hydranth was slowly drawn back and the galvanometer slowly deflected, indicating that the end was becoming more negative. Replacing the glass plate, the hydranth would emerge and the P.D. became positive.

The P.D. between the distal hydranth and the proximal end often showed quick variations in a negative direction, and these were coincident with the contraction of the tentacles. The current was of short duration as contrasted with the negative current caused by contraction of the coenosarc. These "action" currents suggest a reason why, when the stems were measured in air, the distal end was almost invariably negative to the proximal end during the late development of the hydranth, while in moist chambers it was positive during the same period. When the stems were measured in air the distal end

TABLE XXI

POTENTIAL DIFFERENCES IN *Tubularia*

Measured in chamber shown in Figure 3. *AC*, entire stem; *AB*, distal half; *BC*, proximal half. Negative sign indicates that a current will flow from the proximal end of a piece through the galvanometer to the distal end. Readings in microvolts. Temperature: 25° C.

Exp.	Length	Hours after Cutting	1			2			3		
			AC	*AB*	*BC*	*AC*	*AB*	*BC*	*AC*	*AB*	*BC*
1	27–35 mm.	0	−920	−770	− 270	+ 50	+ 40	+ 20	−110	−310	+186
		3	− 30	− 90	+ 160	−100	−130	+ 30	− 20	−100	+ 80
		6	+ 70	− 80	+ 140	− 60	− 90	+ 20	+ 80	− 90	+140
		18	+ 50	−200*	+ 250	− 70	− 90*	+ 30	+120	−100*	+230
		24	+110	−110	+ 250	− 10	− 30	− 30	+160	− 40	+180
		32	+100	−100†	+ 210	0	− 15†	+ 15	+ 50	+ 20†	+ 60
2	33 mm.	0	−270	−100	− 620	−240	−440	0	−200	−100	− 80
		4	−175	−165	− 10	−250	−350	+ 10	−130	−150	0
		17	+210	−170*	+ 350	+100	− 60*	+ 160	− 30	− 60	+ 30
		23	+450	−150	+ 590	+110	−170††	+ 315	− 60	− 95*	+ 35
		28	+150	− 20†	+ 170	+ 50	− 70†	+ 180	+ 5	− 10†	+ 10
		41	+ 90	− 30	+ 110§	+ 50	− 40	+ 110	− 20	− 50	+ 20
3	35–40 mm.	0	−120	+ 60	− 210	+ 30	+ 40	0	−130	− 40	− 40
		1½	+175	0	+ 175	− 40	− 50	+ 10	+170	0	+170
		3½	+230	0	+ 230	− 20	− 40	+ 20	+ 60	− 20	+ 75
		5½	+ 95	−115	+ 220	− 40	− 60	+ 15	+ 85	− 10	+105
		7½	0	−240	+ 240	− 40	− 40	+ 10	+165	− 40	+205
		9½	− 80	−230	+ 180	− 35	− 45	+ 20	+ 95	− 70	+170
		18½	− 60	−150*	+ 100	− 80	−110*	+ 20	− 30	− 90*	+ 90
		23½	− 30	−110	+ 100	+ 10	− 10	+ 30	−100	− 50	+140
		26½	− 30	−110	+ 70	+ 50	+ 35	+ 15	− 30	− 95	+ 65
		29½	+ 20	0	+ 30	+ 30	+ 20	+ 10	+ 90	− 30	+110
		32½	− 65	−110†	+ 40	− 20	− 35†	+ 10	+110	0†	+110
4	40 mm.	0	−280	−110	+ 50	−310	−500	− 80	−630	−220	− 90
		13	−700	−260	+ 530	− 75	−460	+ 390	−190	−650	+410
		26	−220	−410*	+ 160	0	−110*	+ 110	−120	−210*	+ 80
		37	−250	−730	+ 370	+180	− 70†	+ 260	+170	− 40	+240
		39	−450	−570†	+ 350	+350	− 70	+ 410	+ 80	− 90†	+170
5	27–30 mm.	0	+ 50	+ 70	− 20	− 60	− 40	0	−700	−400	−200
		2 min.	+ 90	+130	− 40	+260	+580	− 30	+420	+300	+200
		2½	− 50	− 70	+ 30	+ 40	+ 40	0	−380	−170	−190
		5½	− 90	−110	+ 50	− 10	+210	+ 210	−150	+ 50	−185
		10	− 80	−120	+ 50	+120	−180	+ 380	− 20	− 80	+ 60
		22	+ 20	− 60*	+ 100	+420	−430	+1,030	+230	− 60*	+300
		27	+160	+100‖	+ 90	+110	−290	+ 460	+680	+250	+480
		29½	+ 80	+ 40†	+ 60	+300	−130*	+ 460	+440	+ 50	+420
		35½	+ 50	+ 25	+ 30	+250	−100	+ 360‡	+140	+ 80†	+ 70
		47	+ 50	− 10	+ 70	+120	+ 90†	+ 220§	+ 15	−110	+130§
		55	+ 40	− 20	+ 50	+ 60	− 80	+ 150	+ 20	−210	+220
		72	.....	.....	.....	+ 60**	− 20	+ 80	+150	+ 50	+100
6	30 mm.	0	−110	−160	+ 70	+125	−185	+ 270	−300	−150	− 60
		1½	+140	+ 45	+ 110	− 60	+140	− 170	.....	.....	.....
		4	+ 65	−100	+ 130	+910	−100	+1,000	+180	+ 45	+115
		9	+370	−630	+1,030	.....	.....	.....	+100	−200*	+300
		21	+460	−190	+ 680	+600	−400	+1,020	+400	− 10	+420
		29	+780	+120	+ 680	+430	− 28	+ 700	+210	− 80	+300
		35	+400	+140*	+ 340	variable	.....	.....	+250	+ 60	+190
		46	+430	−380†	+ 790	−790	−570	− 80	+250	+ 20†	+220
		50½	.....	− 70	+ 60	Preparation bad. Discarded.			.....	.....	.....
		52½	+ 80	−130	+ 210‡	.....	.....	.....	+190	− 50	+250
		56½	− 80	+ 10	− 90	.....	.....	.....	+ 70	−100	+160
		69	.....	.....	.....	.....	.....	.....	+160	− 60	+210

* Distal hydranths developing.
† Distal hydranth emerged.
‡ Proximal hydranth developing.
§ Proximal hydranth emerged.
‖ Distal hydranth in act of emerging.
** Hydranth at both ends growing.
†† Action currents from emerging hydranth.

was often positive when the electrodes were first brought in contact with the ends, but soon became negative. This negativity was probably due to the contraction of the coenosarc, and, in fact in some cases the perisarc at the distal end collapsed, indicating that the developing hydranth was drawn back from the end.

II. Potential Differences in *Eudendrium* and *Pennaria*

The stems were prepared as in former experiments. The main stem of a colony was selected, the side branches cut off and pieces 18–25 mm. in length were used. The difference in potential between the distal and proximal end was measured by placing the stem in a moist chamber with the ends immersed in separate containers of sea water. The electrodes were placed in the containers and the measurement taken. Between measurements the stems were kept in running sea water.

The results are not very satisfactory but do indicate the direction of the P.D. in the stem. In all but the first of the following experiments the potential difference between the distal end and the proximal region was first measured by placing the tip of the distal end in one sea-water container and the several millimeters of the proximal region in the other sea-water container. The potential difference between the proximal end and the distal region was measured in a similar manner. Thus, in the following results, the two ends have been measured separately. This was done because the potential difference between two cut ends fluctuates so much.

A. Potential differences in *Eudendrium*

In Table XXII the P.D.'s are recorded during regeneration. In these experiments the two cut ends of a main stem were placed in sea-water cups in paraffin and the electrodes placed in these cups. The measurements are therefore between the two ends.

Nos. 1 and 2 regenerated at both ends. No. 3 regenerated at the proximal end while No. 4 started to regenerate at 16 hours, and then stopped. The distal end of No. 3 is consistently negative, which means that the outgrowth is positive, since the sign is of the distal end. Nos. 1 and 2 are variable, which is to be expected, since regeneration is taking place at both ends. No. 4 is likewise variable and does not regenerate.

In order to eliminate some of the variability which is due to the two cut ends, some experiments were carried out using a slightly different method. In Table XXIII the results were obtained by placing

TABLE XXII

POTENTIAL DIFFERENCES IN *Eudendrium*

Stems 25 mm. long. Readings in microvolts. See text for method of measuring. Sign of distal end, external circuit.

Hours after Cutting	1	2	3	4
0	−550	− 70	−255	0
1	+ 90	−140	−140	− 50
8	− 90	− 50	−410	0
16	− 50†	+250†	−470*	−100*
20	−280	−650	−550	0
42	− 90	+300	−590	− 35
64	− 50†	− 40†	−470*	+150‡

* Outgrowth at *proximal end*.
† Outgrowths at distal and proximal ends.
‡ Outgrowth retracted.

TABLE XXIII

POTENTIAL DIFFERENCES IN *Eudendrium*

Readings in microvolts. See text for method of measuring. The sign refers to the end which is being measured.

	Time after Cutting	1	2	3	4	5
Proximal end	0	−130	+ 290	−580	+420	+480
		+570	+ 490	−570	+700	+300
		+420	.....	+470	.....	.....
	36 hrs.	+150*	+ 500*	+450*	+200*	+200*
Distal end	0	+900	+1,100	+700	+250	negative
		+780	+1,200	+640	+250	negative
		.....	.....	+770	.....	negative
	36 hrs.	+400†	+ 400†	+200†	+180†	+200†

* Proximal outgrowth.
† Distal outgrowth.

the end to be measured in one sea-water cup and immersing the opposite end to the extent of about one-fourth the total length of the piece in the other sea-water cup.

The proximal and distal ends were thus measured separately. The

measurements in this table are thus between the distal end and a proximal level, and between the proximal end and a distal level. When more than one value is given it means that the stem was reversed and a second or third reading taken.

Measured in this way, the outgrowths are almost invariably positive to resting regions. All of the stems regenerate at the distal and proximal ends, and almost all of the outgrowths are positive.

TABLE XXIV

POTENTIAL DIFFERENCES IN *Pennaria*

Readings in microvolts. Stems 18–25 mm. long. The sign refers to the polarity of the end which is being measured.

		Time after Cutting (Hours)	1	2	3	4	5
Exp. A	Distal end	0	−370	−550	−330	−460	− 920
		16	.....	.....	.....	−330*	− 230*
		40	−380*	+660*	−770*	−220*	−1,020*
		70	+400	−400	−200	−250	− 200
	Proximal end	0	+340	+500	+950	+700	+1,140
		16	.....	.....	.....	+690	+ 460
		40	+540†	+970†	+260‡	+550‡	+ 920‡
Exp. B	Distal end	0	−350	−590	−470	−380	− 500
		36	+270†	−530§	+200†	−200§	− 350§
	Proximal end	36	+480†	−730‖	+300†	0‖	0‖

* Distal outgrowth. ‡ Proximal outgrowth. ‖ Growths 1 mm. long.
†No growth. § Growths 1–1½ mm. long.

B. POTENTIAL DIFFERENCES IN *Pennaria*

In *Pennaria*, as with *Eudendrium*, the distal and proximal ends were measured separately to avoid the varying potentials between two cut surfaces. *Pennaria* differs from *Eudendrium* in the size of the outgrowth from distal and proximal ends. In *Pennaria* it is the distal end which usually produces the hydranth, while in *Eudendrium* it is the proximal end.

Table XXIV A shows that the distal cut end is negative and remains so. Outgrowths are visible at 16 hours. No. 1 is positive at 70 hours and No. 2 at 40 hours, but these are the only exceptions.

The proximal cut end is positive to distal regions, and regenerates slowly if at all. At 16 hours Nos. 3 and 4 are still positive, no outgrowths appearing, and at 40 hours all are positive with outgrowths in Nos. 3, 4, and 5. These outgrowths are very small compared to the apical outgrowths.

Table XXIV B gives additional data taken in the same way. The distal end is negative with the exception of Nos. 1 and 3, which do not have outgrowths at 36 hours. The proximal ends were measured at 36 hours. Nos. 1 and 3 were positive with no outgrowths, No. 2 negative with an outgrowth of about the same size as the distal outgrowth. Nos. 3 and 4 showed no P.D. between the proximal end and the distal region, but small outgrowths were present.

DISCUSSION

The potentials in *Tubularia*, although small and variable, may be described in the following manner: The hydranth is negative to the middle and shows negative variations during contraction. The hydranth is usually positive to a cut end or a regenerating end. The distal cut end is negative to the proximal cut end but may become positive during reconstitution. Both the distal cut end and the proximal cut end are negative to middle regions during regeneration, as measured by two different methods.

The potentials in *Eudendrium* are of the same order of magnitude as in *Tubularia*, but the regenerating regions are positive to resting regions. Both the proximal and the distal outgrowths are positive. In the stem of *Pennaria* the distal regenerating end is negative while the proximal regenerating end is positive.

There is thus no coincidence of electrical polarity and organic polarity in different hydroids. Including Lund's data on *Obelia* we find the regenerating end positive in *Obelia* and *Eudendrium* and negative in *Pennaria* and *Tubularia*. In the first paper of this series[3] it is shown that there is no relation between the direction of the current and its effect on the polarity of regenerating stems of different hydroids. An impressed electric current may inhibit at the anode, at the cathode, or at either pole, depending on the current strength.

However, a very interesting relationship appears when we attempt

[3] This journal, pp. 344–64.

to correlate the effect of an applied potential with the normal electrical polarity during regeneration. Referring to Figure 4 the directions of the currents during early reconstitution are shown by means of arrows and the magnitudes in round numbers are indicated. The

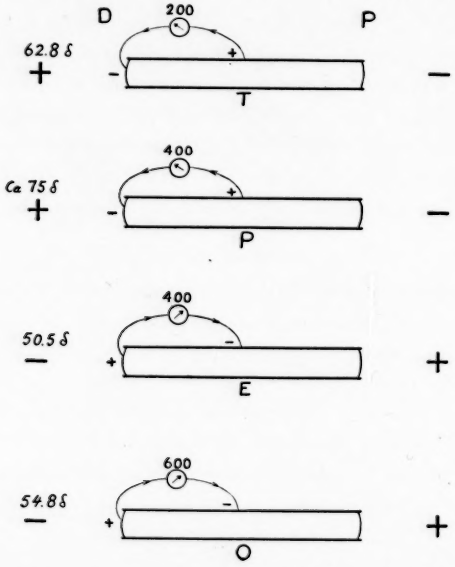

FIG. 4.—Diagram showing the direction and magnitude of the P.D.'s in the stems of *T* = *Tubularia*, *P* = *Pennaria*, *E* = *Eudendrium*, and *O* = *Obelia*, Lund (1925). The magnitude is given in round numbers in microvolts above the circuit in the distal half of each stem. *D* = distal end of stem, *P* = proximal end. The large + and − signs indicate the direction of the current which produces inhibition of regeneration at the distal end of the stem in the diagram. The magnitude of the inhibiting current is given at the distal end of the stem.

large + and − signs indicate the direction of an applied P.D. which will cause inhibition or retardation of regeneration at the distal end. It is interesting to note that the current densities are about the same for all four species, and that the potentials are of the same order of magnitude. In each case it will be seen that reconstitution is inhibited or retarded when the externally applied current opposes the external current of the hydroid stem. In *Tubularia* and *Pennaria*,

where the distal end is negative (in all cases when speaking of the sign of the distal end the sign in relation to the middle of the stem is meant), inhibition occurs at the positive pole. On the other hand, inhibition is obtained at the cathode in *Eudendrium* and *Obelia* where the distal end is positive.

Thus, while during regeneration a bioelectric current may flow externally either to or from the active regenerating region, any interference with this current retards regeneration. Weak currents, 5–8 δ, result in cathodal inhibition in *Tubularia* with perhaps some anodal stimulation.[4] It is found that weak currents decrease the potential difference of stems oriented with the distal end facing the anode, and increase the potential of stems with the distal end cathodal. Strong currents, 62.8 δ, result in anodal inhibition, and these currents reverse the electrical polarity of stems facing the anode. It appears, then, that a slight inhibition is obtained when the normal bioelectric current is increased (cathodal inhibition) and a marked inhibition when the normal bioelectric current is reversed (anodal inhibition).

Child (1924), Child and Hyman (1926), and Hyman and Bellamy (1922), have stressed the fact that the dominant regions of *Tubularia* and *Corymorpha* are negative to resting regions. Hyman and Bellamy (1922), and Child (1924), suggested that this bioelectric current was caused by differences in the rate of metabolism along the axis. Child (1920) further suggested that dominance in general might be primarily a matter of differences in electrical potential and resulting currents.

Lund (1925) calculated that the current required to inhibit regeneration in *Obelia* was of about the same magnitude as the normal bioelectric current. Lund suggests that the normal "electromotive forces developed by the cells themselves act as a means by which adjacent cells may affect each other's orientation and growth."

Obviously we have here a possible mechanism by which the regeneration of one region may be controlled by another region in the hydroid stem. If the hydranth in each species develops at the cut end and sets up a P.D., this P.D. may exert a physiological effect on adjacent regions and exercise control over the behavior of these regions

[4] *Ibid.*, pp. 344–64.

in regeneration. The dominance of the distal end of a stem over the proximal end may then be due to the electrical difference in potential between the two ends, which sets up a current which in turn affects the development of the proximal end. In some cases the effect is merely a retardation of reconstitution of the hydranth as in *Tubularia*, while in *Obelia*, (Lund, 1924), *Eudendrium*, and *Pennaria* the retardation is so great that hydranth formation may be inhibited.

The greatest difficulty in applying this sort of explanation is the difference which we have found in different hydroids. In *Tubularia* and *Pennaria* the inhibiting current must flow in one direction along the stem while in *Eudendrium* and *Obelia* it must flow in the opposite direction. Similarly, regenerating regions in the two groups give rise to currents which flow in opposite directions along the stems.

Another difficulty presents itself when we attempt to show the path of the current in the stem. While a current may be obtained when the stem is out of sea water by placing electrodes on the two ends, we may question whether the current flows in the tissue during normal regeneration in sea water. Almost any two surfaces are dissimilar electrically, and the magnitudes of these differences in the hydroid stem are small. The stems normally are surrounded by a good conductor, sea water, and we may well ask: What are the paths of the current under such conditions? And, also, Does any current at all flow in the tissues?

At the same time it is highly improbable that the direction of the bioelectric current and the direction of the inhibiting current would be opposed in all four cases merely through coincidence. The bioelectric current, if it flows continuously during reconstitution, probably has a physiological effect on regeneration. The marked effect of weak continuous currents on regeneration has been adequately demonstrated by the work of Lund and the writer. If the hydroid stem produces such weak currents continuously it is probable that they have similar effects upon reconstitution. It is intended here to point out only the general relationship of the inhibiting current to the normal bioelectric current.

SUMMARY

1. The electrical potential differences between various regions of the stem of *Tubularia* were measured with the following results: The hydranth is negative externally to regions along the stem. The regenerating region is negative to the middle of the stem. When stems are cut into halves, the distal half has a higher potential difference than the proximal half. When the stem is cut into thirds the distal third has the highest P.D., the middle third shows less P.D., and the proximal third gives the smallest P.D.

2. The regenerating end of the stem of *Pennaria* is usually negative to regions along the stem.

3. The regenerating end of the stem of *Eudendrium* is usually positive to regions along the stem.

4. The relation between the electrical differences of potential of the stem and the inhibiting effect of an externally applied current upon regeneration is shown.

LITERATURE CITED

CHILD, C. M. 1920. Physiological isolation by low temperature in *Bryophyllum*. Bot. Gaz., **70**:249.

———. 1924. Physiological foundations of behaviour. New York.

CHILD, C. M., and HYMAN, L. H. 1926. Studies on the axial gradients in *Corymorpha palma*. Biologia Generalis, **2**:355.

GELFAN, S. 1927. The electrical conductivity of protoplasm and a new method of its determination. Univ. Calif. Publ. Zoöl. **29**:453.

HYMAN, L. H. 1920. The axial gradients in Hydrozoa, III. Experiments on the gradient of *Tubularia*. Biol. Bull., **38**:353.

HYMAN, L. H., and BELLAMY, A. W. 1922. Studies on the correlation between metabolic gradients, electrical gradients and galvanotaxis. *Ibid.*, **43**:313.

LUND, E. J. 1922. The Normal electrical polarity in *Obelia*. A proof of its existence. Jour. Exp. Zoöl., **36**:477.

———. 1924. Experimental control of organic polarity by the electric current. *Ibid.*, **39**:357.

———. 1925. Experimental control of organic polarity by the electric current. V. The nature of the control of organic polarity by the electric current. *Ibid.*, **4**:155.

MATHEWS, A. P. 1903. Electrical polarity in the hydroids. Amer. Jour. Physiol., **8**:294.

THE EFFECT OF CAFFEINE UPON THE CARDIAC RHYTHM OF ADULT FISH HEARTS

(Five figures)

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THE author in a previous report (1932) has shown that caffeine has no apparent effect upon the cardiac rhythm of fish embryos prior to innervation by the vagus: the initial response of the innervated heart to the drug is characterized by frequent pauses and a decided irregularity in the rhythm. This condition changes in a few minutes to a constant rate, which is much slower than normal. A review of the literature reveals numerous papers on the effect of caffeine upon the amphibian, turtle, and mammalian hearts, but only one paper, by Beresin (1913), appears on the fish. Beresin worked on isolated, adult fish hearts, and as his results are not in agreement with the results obtained on the embryonic heart, it was thought advisable to restudy the effect of caffeine upon the adult fish heart. As this heart is probably not innervated by the sympathetic system, it is a simpler mechanism than the frog, turtle, or mammalian heart, which have a double innervation. A study of the effect of caffeine upon this simpler structure may also throw some light upon the general question of the specific effect of caffeine, which is somewhat confused in the literature, upon the heart mechanism.

MATERIALS AND METHOD

Individual fish varying in length from five to eight inches were found well suited for these experiments. The following species were used: wall-eyed pike, *Stizostedion vitreum* (Mitchill); black crappie, *Pomoxis sparoides* (Lacepede); carp, *Cyprinus carpio* (Linn); chub, *Semotilus a. atromaculatus* (Mitchill);[1] and a shiner, *Notropus cornutus frontalis* (Agassiz).[1] These fishes were collected as needed in local waters during the early spring and kept in an aquarium in the laboratory for a few days to allow them to become acclimated to room temperature before being used. They never remained in the laboratory

[1] Identified by Carl L. Hubbs.

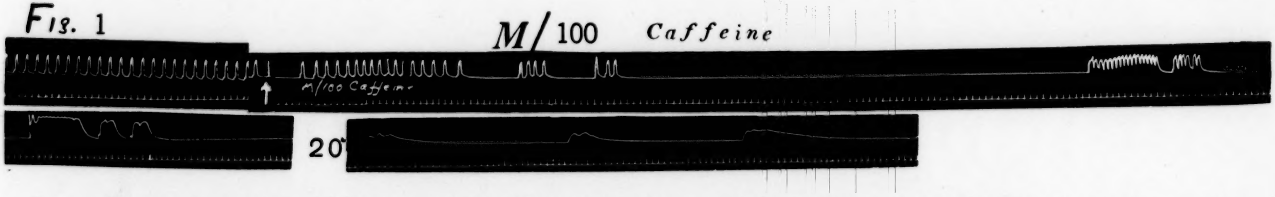

Fig. 1.—Kymograph record of a fish heart treated with M/100 caffeine solution. The arrow indicates the time of application of caffeine. The figures represent the time elapsing between records and the lower reading is a continuation of the upper record. Time marker indicates seconds.

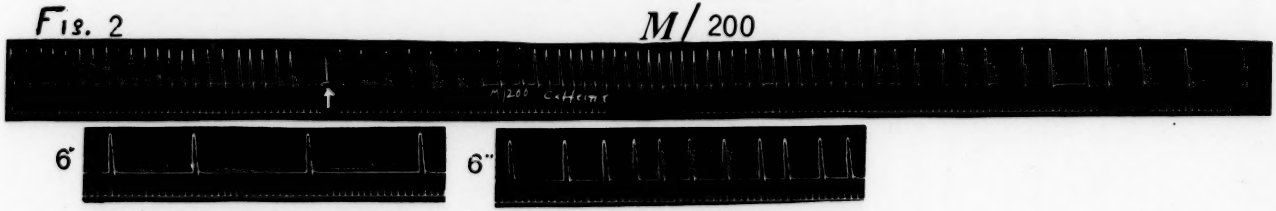

Fig. 2.—Record of a fish heart treated with M/200 caffeine solution

for more than 10 days. In preparation for these experiments, the fish were pinned with the ventral side up in a glass tray of 1,000-cc. capacity containing a layer of paraffin and beeswax 1 inch thick. A trough was cut into the wax to receive the fish. Frog Ringer's solution was poured into the tray in sufficient quantities to cover the fish to a depth of $\frac{1}{2}$ inch. A small incision in the ventral surface of the fish was made anterior to the pectoral fins in order to expose the heart in the pericardial cavity. The point of a very fine needle was carefully inserted into the muscular wall of the apex of the ventricle. Care was taken not to puncture the cavity which would allow the blood to escape. The pin was attached to a heart lever and a record was made on a kymograph. It was found necessary in order to maintain the regular heart rhythm during the experiment, to administer artificial respiration by directing a stream of air bubbles from an air pump over the gills. The gill movement ceased within a few minutes, but the blood continued to flow through the heart and the cardiac circulation continued for 2 or 3 hours. A normal record was taken of each heart prior to the addition of caffeine to the solution in the tray in which the fish was immersed and a continuous record was taken during the entire experiment.

RESULTS

Caffeine.—The reaction of the adult fish heart to caffeine is dependent upon the concentration used. Within a few seconds after treatment with M/100 caffeine solution (Figure 1) the heart quickly comes to a standstill after a few somewhat irregular beats. The heart remains in a diastolic condition for several seconds and is engorged with blood. The heart then resumes beating at a greatly accelerated rate and goes into a state of partial tetany, which is immediately followed by a quiescent period of several seconds' duration, and again follows a series of tetanic contractions. Finally the rhythm becomes regular but much slower than normal. There seems to be no differences in the force or amplitude of the beat after treatment with caffeine. In the case of a more dilute solution of caffeine, M/200 (Fig. 2), the heart does not approach the state of tetany, but the rate is very much reduced after treatment with the drug. Within a few seconds after immersion in caffeine M/200, the rate becomes much slower, often several seconds elapse between beats. The am-

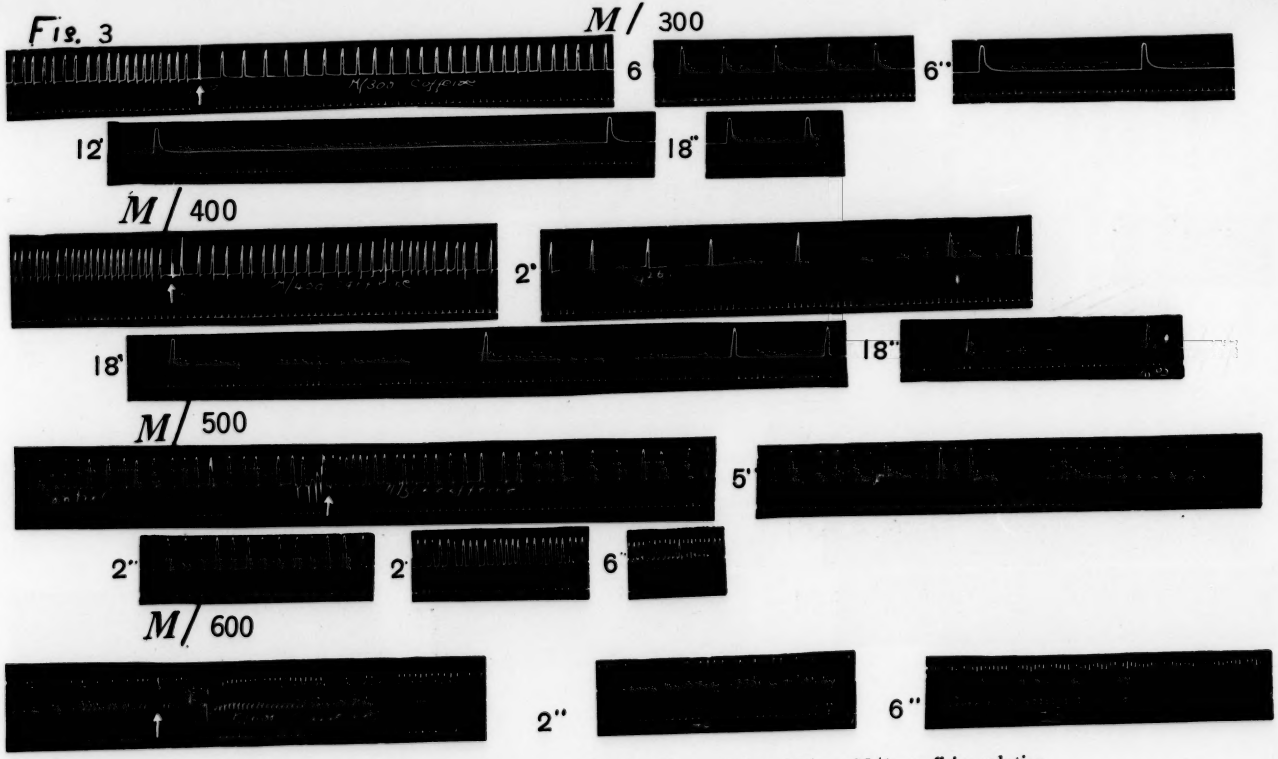

FIG. 3.—Shows four records of hearts treated with M/300, M/400, M/500, M/600 caffeine solution

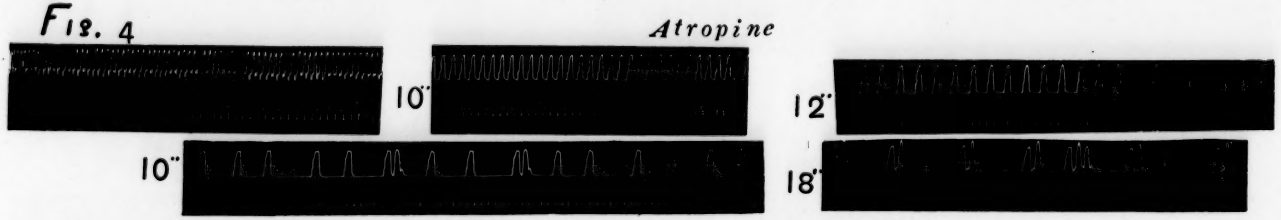

Fig. 4.—Record of a fish heart treated with atropine sulphate

Fig. 5.—Record of a fish heart treated with M/100 caffeine solution after atropine injection

plitude remains the same but there is a noticeable slowing during the relaxation phase, and thus the curve of a single beat is considerably widened. As the concentration of caffeine is gradually reduced from M/300 to M/600 (Fig. 3) the reduction in the rate of heart beat, after caffeine, is gradually decreased; the time interval between beats is gradually shortened as the concentration of caffeine is lowered to a concentration of M/600, in which case the effect of caffeine is evident only by the presence of a compensatory pause, occurring at rather definite intervals after every five or ten beats of the heart. In all cases the heart rate gradually increases after a longer exposure, but returns to normal only in concentrations of M/500 and M/600, where the rate after an exposure of 30 minutes increases and surpasses the normal. Concentrations of caffeine more dilute than M/600 show no effect upon the heart.

Atropine.—In order to distinguish between the effect of caffeine upon the nerve supplying the heart and the cardiac muscle, a series of experiments was conducted by injecting 1 cc. of a 1 per cent atropine sulphate solution (Fig. 4), which paralyzes the vagus terminations, into the body cavity of a fish before the pericardial cavity was opened and the heart exposed to the effect of caffeine. Figure 5 shows a record of a heart which had been freed from the controlling influence of the nerves by injection with atropine prior to immersion in M/100 caffeine solution. It may readily be seen from the record that after the addition of caffeine the amplitude is decidedly increased, but the rate is slightly reduced for several seconds, then follows a series of very rapid pulsations terminating in a compensatory pause. This record is decidedly different from that shown in Figure 1, in which case the heart was under the control of the vagus. The effect of a dilute solution of caffeine, M/500 following an injection of atropine apparently has no noticeable effect upon the rate or amplitude. A series of experiments in the reverse order were conducted as follows: The fish were opened and immersed in M/100 caffeine solution, and after the initial stoppage of the heart the fish was injected with 1 cc. of 1 per cent atropine solution. Within 1 or 2 seconds the heart resumed beating at an increased rate and the record follows very closely Figure 5, in which case caffeine followed atropine.

Isolated heart.—A series of experiments were conducted on the isolated fish heart. The heart was carefully removed from the fish by

cutting the aorta at the junction of the bulbus arteriosus and the veins were severed at their junction with the sinus. The bulbus was pinned to a paraffin-beeswax layer in the bottom of a Petri dish filled with Ringer's solution. The apex of the ventricle was fastened to a heart lever and a record of the ventricular beat was recorded. The results obtained substantiate very closely those with atropine. After addition of M/100 to M/400 caffeine, the amplitude was increased, but the rate was reduced about 50 per cent. An M/500 caffeine solution produces no effect upon the isolated fish heart.

CONCLUSIONS

It appears from a study of the graphs on fish hearts that the effects of caffeine upon the cardiac mechanism may be twofold: (1) a stimulation of the vagus supplying the heart, and (2) an effect on the irritability on the cardiac muscle. The long quiescent period following treatment with M/100 caffeine is probably due to a stimulation of the vagus and at the same time caffeine is increasing the irritability of the cardiac muscle to the inner stimulus, so that finally the irritability of the heart muscle offsets the depression by the vagus and the heart escapes from the nerve, resulting in a series of very rapid contractions. The contractions occur so rapidly that the heart has no time to recover from the previous beat; thus a state of incomplete tetany is obtained, which approaches tetany as nearly as it is possible to obtain it in the cardiac muscle. The amplitude of contraction in an innervated heart is not changed. A compensatory pause occurs after a series of these rapid beats, during which time the heart relaxes more completely than normal and recovers from the toxic materials produced during the action. The cardiac muscle is irritable to direct electrical stimulation during the quiescent period while it is held in control by the vagus. The contraction is of the same magnitude as the normal beat. Therefore, it is evident that the contractility of the cardiac muscle has not been effected by the drug. The control of the vagus over the heart can be demonstrated by electrical stimulation of the nerve, which caused the heart to stop beating for 2 or 3 seconds, but it is impossible to hold the heart at a standstill for a longer period of time by electrical stimulation.

In the case of a more dilute solution of caffeine, M/200–M/500, the effect upon the nerve is evident by a gradual slowing in the rate and a gradual lengthening of relaxation phase, but there is no evi-

dence of tetany as is found in higher concentrations. After an exposure of 15 minutes or longer the heart apparently escapes from the vagus and the rate is increased, but never reaches normal except in the concentrations of M/500 and M/600. The increase of irritability of the cardiac muscle is less marked than in the case of M/100. This may be due to the controlling action of the vagus suppressing the stimulating effect on the muscle. At a lower concentration, M/600, the effect of the vagus does not seem to be evident, but the increase in irritability is indicated by a series of rapid contractions preceding a compensatory pause. Solutions of M/700 and M/800 appear to show no effect upon the vagus or cardiac muscle.

Further evidences on the differential effects can be obtained from a study of Figure 5, where the heart was freed from the action of the vagus by atropine, and in isolated hearts where the heart was removed from the fish and immersed in M/100 caffeine solution. In either case the long stoppage of the heart is not evident, but increased irritability of the cardiac muscle is decidedly marked by powerful contractions, which are not evident when the heart is under the control of the nerve. After an exposure of a few minutes the rate is again increased for a few beats, but is held in check by the compensatory pause. However, the heart never goes into a condition of incomplete tetany as in the case of the innervated heart. The condition of tetany is perhaps prevented in the denervated heart by the frequent appearance of the compensatory pause. A dilute solution of M/500 seems to have no effect upon irritability of the cardial muscle.

SUMMARY

The results of the experiments on the effect of caffeine upon the adult fish hearts indicate that caffeine in solution may reduce the rate of the cardiac rhythm by its effect upon the vagus supplying the heart, and that caffeine in the concentrations used decidedly increases the irritability of the cardiac muscle, making it more sensitive to the inner stimulus.

LITERATURE CITED

BRINLEY, F. J. 1932. A physiological study of the innervation of the heart of fish embryos. Phys. Zoöl. **5**:527.

BERESIN, V. I. 1913. Über den einfluss der gifte auf das isolierte Fishchterz. Arch. ges Physiol., **150**:549.

THE RELATION OF NUMBERS OF PARAMECIUM CAUDATUM TO THEIR ABILITY TO WITHSTAND HIGH TEMPERATURES[1]

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THE possibility of beneficial effects of crowding of animals has been the subject of considerable recent experimental work. These effects may be of two sorts: first, those involving a differential in the rate of reproduction or growth; and, second, those which change the ability of the animals to survive unfavorable conditions. Robertson (1921*a*, 1921*b*, 1924*a*, 1924*b*) found that, under certain conditions grouped Infusoria reproduced more rapidly than isolated individuals. He assumed the presence of a substance given off by the animals during fission into the medium, which had an accelerative effect on the rate of reproduction. He proposed the term "allelocatalysis" for the phenomenon. Robertson's work has been variously confirmed and disputed by succeeding investigators. Allee (1931, 1934) has reviewed the literature dealing with this subject quite fully and critically.

In the matter of group protection against unfavorable conditions, Robertson (1924*a*), working with *Enchelys farcimen*, made the following observations with respect to numbers of animals in relation to resistance to high temperatures:

> Temperatures above 30° C. prevent subcultures of single individuals of *Enchelys farcimen* from multiplying and the isolated individual at this temperature almost invariably dies. Cultures initially containing a large number of individuals, however, will multiply and survive continued exposure to considerably higher temperatures. Individuals isolated from such cultures and kept at the same temperature invariably die. Hence the contiguity of many organisms affords mutual protection against the adverse effects of high temperature.

Robertson assumed that the same substance which he postulated as the cause of acceleration in the reproductive rate of grouped animals over isolated ones served also to protect grouped animals against

[1] The writer acknowledges his gratitude to Dr. W. C. Allee for suggesting this problem and for giving aid and advice in its investigation.

high temperatures. There is no record that he went further in the analysis of this phenomenon. Drzewina and Bohn (1926, 1928) reported group protection of sperm against high temperature and also assumed the secretion of a protective substance.

The resistance of *Paramecium* to high temperatures has been studied by Hutchison (1913, 1915), who found that the resistance varied among races and in relation to the salt content of the medium; that the resistance was increased with an increase of the salt content of the medium; also, that heat resistance was not affected perceptibly by the age of the culture and its accompanying changes, nor was it affected by the ordinary changes in the acidity of the medium. Working with a more refined technique, Chalkley (1930), while investigating the mechanism of death in *Paramecium*, found a close correlation between the pH of the medium and the time required to kill the animals when suddenly exposed to a temperature of 40° C. He reported that the curve of resistance, with the pH varied, is bimodal, with a high resistance at pH 6.8, a maximum at pH 8.0, and a lesser resistance between these points, at pH 7.2. He also reported that an excess of sodium salts greatly reduced the resistance time; that an excess of potassium salts reduced the time in the acid range but increased it in the alkaline range; and that an excess of calcium salts increased the time in the acid range but reduced it in the alkaline range.

The conclusions of Hutchison and Chalkley, as noted above, were approximately diametrically opposed. It seems quite possible, however, that Hutchison's technique was not sufficiently refined.

In relation to varying temperatures a number of bimodal curves have been shown to exist. Heilbrunn (1924) found that the effect of temperature on the viscosity of protoplasm is expressed in this way. Earlier, Plough (1917) had found a similar situation with respect to the phenomenon of crossing-over in *Drosophila*. Brown and Banta (1932) found that a bimodal curve was required to express the effect of temperature on the production of males of *Moina macrocopa*.

MATERIALS AND GENERAL METHODS

Since a number of workers following Robertson had questioned both the phenomenon of the acceleration of the division rate of

groups of Protozoa over isolated individuals and also his explanation of it, it seemed advisable to reinvestigate the matter of group protection of Infusoria against lethal temperatures. *Paramecium caudatum* was chosen for this purpose. The animals were maintained in standard hay infusion. This was prepared by boiling 5 grams of timothy hay in a liter of tap water for 10 minutes and then straining the liquid through flannel. The infusions were inoculated with bacteria from previously made cultures. They were seeded with *Paramecium*, usually 4–6 days after the bacteria were introduced. The animals were maintained in as homogenetic a condition as possible by starting new isolation cultures from individuals taken at random from the clone. These re-isolations were made at intervals of not more than 2 weeks. In earlier experiments ½ liter and 1-liter stock cultures were used, while in the later experiments 2-liter and 10-liter stock cultures were maintained. During the times of the year when these stock cultures could be maintained at little variation in temperature in the laboratory they were kept on the laboratory tables. For these, temperature variations were recorded. When, however, weather conditions became unsettled, the cultures were removed to inside rooms, where the temperature fluctuation did not exceed 3° or 4° C. In some of the later experiments the stock cultures were kept under constant temperature conditions. A few exceptions to this procedure will be pointed out later. In the experiments for maintaining high, constant temperatures there were used, at various times, two Freas water baths, two specially constructed constant-temperature rooms in Whitman Laboratory at the University of Chicago, and a specially devised water bath which maintained a constancy of temperature to within 0.15° C. The most critical of the experiments were performed using this apparatus. Finely drawn pipettes were used for handling the animals. Counts and other examinations were carried on by means of a binocular dissecting microscope. Depression slides of various capacities from ½ to 2 cc. were used for isolation and small group cultures. These were kept in 9-inch covered glass dishes. Mass culture experiments were made in glass jars. The sizes most commonly employed were of 2-liter and 10-liter capacities. Ordinary bacteriological culture tubes were used to contain samples of large mass cultures in the later experiments where samples were

employed to ascertain conditions in large cultures. Populations of cultures were taken by direct count in the case of depression-slide cultures; they were approximated in the case of mass cultures by counting the populations of measured samples, a procedure employed and evaluated by Jahn (1928). In counting large numbers of individuals in a small area, they were drawn slowly into a capillary pipette and counted as they entered. This method was found to be satisfactory for sample counts but not for counts when the animals were to be used again, as usually some of the individuals would stick to the side of the pipette, so that the number expelled from the pipette would be considerably less than the number taken in. Records were kept of the age and pH of culture media and the age of clones used in the experiments. In the earlier experiments pH readings were taken by means of a Youden potentiometer. For the later ones, Hynson, Wescott, and Dunning and Hellige colorimetric sets were used.

EXPERIMENTS

A. RESISTANCE OF SMALL GROUPS AND ISOLATED ANIMALS

Robertson's original observations on the relation of age of culture to resistance to high temperatures had been with mass cultures as they had been kept in his laboratory during the extremely hot weather that sometimes prevailed. He found that sparsely populated (i.e., freshly seeded) cultures showed less resistance to heat than densely populated ones; also, that isolated individuals showed less resistance than members of dense populations. He did not record whether the isolations were made into the same medium in which the animals had been living or fresh medium. Few other data were recorded. Peterson and others who attempted to repeat other phases of Robertson's experiments usually used isolation or small group cultures in depression slides as a means of determining the behavior of individuals. In order to conform to this type of approach in the analysis of the resistance of *Paramecium* to high temperatures, the depression-slide technique was employed in the earlier experiments. The animals were placed in plate-glass slides which contained depressions of approximately 2 cc. capacity. In a single series five animals were placed in one slide with a given amount (usually 1 cc.) of infusion and five other animals were placed one each in five other

slides containing equivalent amounts of medium. The volume of medium, the kind and condition of medium, the treatment of the animals with respect to washing, and the temperature were varied in different experiments. The bacterial growth varied widely among the cultures of an experiment; and there were marked variations in the shape of the mass of medium on the depression slide. These affected the rate of evaporation and the ratio of glass surface in contact with the medium to the volume of the medium. The latter is about three hundred times as great in a depression slide as in a large stock culture. The glass surface ratio might affect the depression-slide cultures in two ways; first, by removing from the culture bacteria and bacterial products, as shown by the formation of a slime of zoögloea on the glass in contact with the medium; and second, perhaps, by throwing into the culture a solution of the glass substance. Many workers have shown that slime and mucous secretions affect media in which animals live by adsorbing certain substances from them. Carpenter (1927) showed that fishes can remove considerable quantities of lead nitrate from solution by adsorbing it in the mucus on their bodies. Allee and Bowen (1932) accounted quantitatively for the removal of colloidal silver from a suspension by goldfishes in a similar manner. It is impossible to say at present to what extent or in what way the zoögloea on a depression slide adsorbs any substance which might affect the resistance of *Paramecium* to high temperatures. With respect to the possibility of some of the glass substance becoming dissolved in the medium, see Kalmus (1929, 1931). The depression slides were made of hard plate glass and were thoroughly cleaned and soaked for long periods in distilled water as a means of removing all soluble substances. General observations indicate that the zoögloea relationship is more important in the present experiments than is the solution of materials from glass.

Later studies showed that the successional cycle in pH was carried as far in 12 hours in 1 cc. cultures as it was carried in 2 weeks in much larger cultures. The variable results noted in the foregoing experiments, due to the variable factors just given, were magnified still further by the action of the high temperature in speeding up the reproductive rates of both the Paramecia and the bacteria. Therefore,

this type of experiment was abandoned in favor of others in which the cultures developed more slowly and which could be more nearly completely controlled.

B. RESISTANCE IN MASS CULTURES

1. *During normal development of cultures.*—Jahn (1929) studied the reproductive rate of *Euglena* sp. in relation to the density of population of the culture. He used a mass-culture method. He estimated populations by counting the organisms in small samples from the cultures, as a substitute for the absolute counts of the numbers of organisms in depression-slide cultures, which had been used customarily. He believed that sample counts from large cultures gave a more accurate representation of the normal conditions of cultures than the counts from slides where the life-conditions are far from normal. Many other workers have pointed out that isolation and other small cultures on slides fail to give an accurate picture of the normal life-processes of microörganisms. In the course of the experiments described in the preceding section it was seen that both at high and at room temperatures the large stock cultures of *Paramecium* carried on much better than did cultures in depression slides. Mass cultures were frequently found with population densities as great as 500 animals per cc., a number that never was approached in 1 cc. cultures. Jones (1930) has made similar observations.

Chalkey (1930) removed small numbers of Paramecia from mass cultures and exposed them to lethal temperatures under varying conditions of pH as a method of studying the correlation between resistance to heat and the pH of the medium. His experiments suggested, as a means of determining the variations in heat susceptibility at the different stages of a culture cycle, the possibility of maintaining the organisms in large stock cultures at moderate temperatures, while checking their population changes by means of sample counts and measuring their resistance to high temperature through the use of sample animals taken from the large group.

After obtaining only negative results from depression-slide cultures, it was decided to attempt an adaptation of Jahn's and Chalkley's techniques for the study of the resistance to high temperature in *Paramecium*.

The initial experiments were carried out as follows: Several series of mass cultures were set up. A single series was made as follows: A 10-liter quantity of standard bacterized hay infusion was made and allowed to stand for 1–4 days. Individual cultures were made from this by straining the infusion through flannel and placing 2 liters in each of four 4-liter jars. In order to make the medium as nearly identical as possible in the four jars, it was stirred frequently during the measuring-out process. Animals were obtained for mass seeding from a rapidly dividing culture. They were washed by mild centrifuging, so that their original medium was diluted at least five hundred thousand times. Then they were further diluted with medium into which they were to be placed until they gave consistent population counts of approximately 100 per cc. One jar of each series was left unseeded to serve as a control for the normal bacterial effects; the other three were seeded with the freshly washed animals, so that they had an initial population of approximately 0.05 animals per cc., 0.2 animals per cc., 0.8 animals per cc., respectively. It is noted that each succeeding concentration is four times that of the culture immediately preceding.

Observations were made at intervals of 24 hours. The pH of the blank and the three inhabited jars was recorded. The media were stirred thoroughly, and 1-cc. samples were taken out for counting the population. With the population less than 50 per cc., ten 1-cc. samples were counted; with the population from 50 to 200 per cc., five counts were made; with the population over 200 per cc., three counts were made. The average of these counts was computed, and this figure was taken to represent the population density of the culture for that time. It is believed that this method gives a close approximation to the actual number of animals present.

In order to determine the resistance to heat, after considerable experimentation with other temperatures, a temperature of 40° C. was used. This temperature was found to have the advantages previously determined by Chalkley (1930), especially since the death time ranged from 10 to 20 minutes. The significance of this particular temperature will be discussed later. In making the resistance test, 1 cc. of the culture medium containing the average number of organisms was placed in each of five ½-inch bacteriological culture

tubes. These were placed, at 2-minute intervals, in a water bath heated to 40°. In the earlier experiments the tubes were paraffined as an additional precaution against the operation of any factors associated with the glass. Parallel tests with paraffined and unparaffined tubes soon showed that the practice of coating the tubes with paraffin made no difference in the resistance time and so it was abandoned. This finding coincides with that of Johnson (1933), who investigated the desirability of coating depression slides with paraffin in the study of reproductive rates in *Oxytricha*.

The tubes were taken out of the bath at the end of a period previously determined as one which would indicate the death time. They were then cooled for 20 minutes by standing in running water at a temperature of about 20° C. and poured onto depression slides for examination. The actual numbers of animals in the samples taken for the tests varied within the normal range for random sampling. Under some of the conditions of the experiments the animals disintegrated under the influence of the heat. These factors made it impossible to use the time of survival of 50 per cent of the organisms as an index of their heat-resisting ability, as would be customary in an experiment of this sort. Observations on the death time showed that only rarely did a particularly tenacious animal survive much longer than the rest of the group. Therefore, that exposure interval at which the last signs of life were detectable was taken as the index of heat resistance.

Table I gives a summary of the conditions of pH, density of population, and survival time against a temperature of 40° C. in relation to the age of the cultures, for one of five series run under comparable conditions. Examination of the data from these experiments permitted a number of generalizations which were useful in their further analysis.

As reported by many previous workers, a typical hay infusion has a pH value, when freshly made, of about 7.4. In about 2 days this value has dropped to around pH 6.0. Then the value rises through the next 3 or 4 days to the initial level. After this there is a further gradual increase to about pH 8.0, near which point the pH remains throughout the rest of the history of the culture. The exact values for the points on a curve plotted to show this trend vary with the

nature of the water used, with the size of the cultures, and probably with the nature of the bacterial flora. The pH cycles in cultures used in some of the later experiments which were made up from water much richer in calcium carbonate averaged about five-tenths of a pH unit higher at comparable stages in the progression. The cultures of one series were made from freshly boiled culture medium. The first part of these culture histories was passed through under conditions of low pH. The other series of cultures were inoculated after

TABLE I

HEAT RESISTANCE OF *Paramecium* IN RELATION TO DENSITY OF POPULATION AND PH OF CULTURE

The "A" cultures were left unseeded with animals in order to show the normal pH cycle of cultures without Infusoria.

Age of Culture in Days	Series X6										
	Population				pH				Resistance to 40° C. in Minutes		
	A	B	C	D	A	B	C	D	B	C	D
0	0	0.05	0.2	0.8	6.60	6.60	6.60	6.60	.....	.....	.....
1	0	0.4	1.3	5.5	7.15	7.11	7.11	7.11	.....	20	20
2	0	4.2	10.7	47.5	7.10	7.11	7.20	7.20	21	21	21
3	0	27.4	73.8	210.0	6.94	7.10	7.26	7.26	16	14	13
4	0	120.3	212.0	431.3	6.99	7.20	7.26	7.24	15	15	14
5	0	402.7	415.3	436.3	7.26	7.37	7.37	7.33	11	10	10
6	0	451.0	428.0	436.3	7.34	7.37	7.45	7.45	15	16	18
7	0	465.0	426.0	443.5	7.61	7.53	7.59	7.59	22	21	22

the pH had dropped to the minimum and had started back to higher levels.

The pH cycle of the cultures runs through practically the same course and at the same rate, regardless of the density of the initial seeding with Paramecia or even the lack of seeding. Table II shows this relationship. These figures tend to contradict the conception expressed by Darby (1930) and others that protozoan populations affect materially the pH of their cultures. Darby, using *Paramecium aurelia*, *P. caudatum*, and *Stylonichia pustulata*, suggested that each organism tends to buffer its medium so as to bring it to its optimum pH, and that the more animals put in at a time the more quickly this

is brought about. It seems possible that in placing large numbers in the medium, as contrasted with small numbers, he may have carried over larger quantities of bacteria, so that the pH cycle which depends on bacterial growth naturally would be accelerated. The bearing of this question on the present problem will be discussed later in this paper.

The populations of the different cultures within a series were existing under quite similar environmental conditions at any given time. This was indicated by the close correspondence in pH values noted above, and by the fact that the populations of the cultures in a single series tended to reach approximately equal maximum concentrations

TABLE II

THE pH CYCLE IN UNPOPULATED MEDIUM COMPARED WITH CYCLES IN POPULATED MEDIA

(Based on data of which those shown in Table I are typical.)

	Culture Series					
	X_4	X_5	X6	X_7	X8	Total
Number of times blank culture has lower pH than the populated cultures of the same series	12	8	13	2	3	38
Number of times blank culture has same pH as the populated cultures of the same series.....	9	7	4	4	6	30
Number of times blank culture has higher pH than the populated cultures of the same series	6	0	3	13	6	28

irrespective of the density of the original seeding. Myers (1927) made similar observations with respect to population maxima in cultures of *Paramecium*.

The daily reproductive rate varied widely among the various cultures and in the various stages of the same culture. The daily reproductive rates may be expressed by "reproductive quotients," obtained by dividing the number of organisms in a unit volume of the culture on a given day by the number in a corresponding volume on the preceding day. This represents the average number of progeny of a single animal for the given period. The reproductive quotients in this series of experiments varied between 1 and 10. The highest values were found in the earlier stages of the cultures but

occurred in populations up to 200 animals per cc.; e.g., the quotient for the "A" culture in Series X_7 for the fifth day, where the population leaped from 29.9 to 203.7, is approximately 7.

In addition to the foregoing general observations based on data shown in part in Table I, the data were analyzed further to determine whether there was a correlation between the survival time of the organisms and any other recognizable factors. In order to group results into larger units, the survival time was classified as follows: 8–12 minutes were classified as "short time"; 13–16 as "medium time"; 17–21 as "long-time." On this basis the survival time was short in fourteen instances, medium in twenty-five instances, and

TABLE III

SURVIVAL TIME OF *Paramecium* AT TEMPERATURE OF 40° C. IN RELATION TO AVERAGE REPRODUCTIVE QUOTIENTS

(Based on data of which those shown in Table I are typical.)

	No. of Instances	Average Quotient	Maximum Time in Minutes	Minimum Time in Minutes
Short time (8–12 min.)	14	4.4	10	1
Medium time (13–16 min.)	25	4.0	8	1
Long time (17–21 min.)	21	3.6	10	1

long in twenty-one instances. Table III gives these data in relation to the average reproductive quotients for each group.

On account of the extremely wide spread of the numbers from which the average quotients are computed, these figures have no statistical significance. Nevertheless, it is of interest to note that the longest time of heat resistance is associated with the smallest average quotient of reproduction. If, as is usually supposed, the fission rate is a measure of metabolic rate, then, according to the theories of Child (1915), the more slowly dividing animals would be expected to survive sudden exposure to relatively high temperatures longer than those which divide more rapidly. Although these figures are not statistically significant in themselves, they will be referred to later as indicating a possibly modifying effect on other factors.

In correlating the survival time with the population per cubic centimeter, it was difficult to establish a proper basis for grouping

populations. On a somewhat arbitrary basis, however, the populations were classified as "sparse" when the organisms numbered less than 10 per cc., "medium" when they ranged between 10 and 80 per cc., and "dense" when they ran over 80 per cc. Table IV shows this correlation. Here, again, the wide scatter of the numbers from which the averages are obtained, render them of little statistical significance except in the comparison between the sparse and dense populations, where the latter are shown to have a decidedly longer survival time.

A third type of correlation capable of analysis from such data as shown in part in Table I is that between the pH of the cultures and

TABLE IV

POPULATION PER CUBIC CENTIMETER OF CULTURES IN RELATION TO SURVIVAL TIME

(Based on data of which those in Table I are typical.)

	No. of Instances	Average Time of Survival in Minutes
Sparse (less than 10 per cc.)	19	12.5
Medium (10–80 per cc.)	31	13.2
Dense (over 80 per cc.)	32	16.0

the survival time of the populations. The survival times for the various pH values were averaged, with the results shown in Table V. It is seen that the curve of resistance is roughly bimodal. Chalkley (1930) found that the resistance to a temperature of 40° C. by *Paramecium caudatum* varied with the pH of the medium in which the animals were exposed; the curve here also was bimodal, with high points at pH 6.8 and 8.0. He also found that the loci of the curve were modified by excesses of various salts in the medium and that slight departures from normal in the chemical content of media exert strongly modifying effects on the resistance time of the animals. Since a curve based on present data would be still more of a departure from Chalkley's normal curve, it may be assumed that there are factors not yet analyzed that also modify the reaction.

Examination of Tables II, III, and IV shows that the conditions conducive to greatest survival are furnished by cultures which have

attained a dense population of animals with reproduction markedly slowed up, and which have progressed to a relatively high and stable pH.

The content of an old culture medium differs from that of a new one in several important respects: in the numbers and species of the bacteria, in the numbers of Paramecia, and in the excretion products of the bacteria and the Paramecia. Allee (1931) has shown, using *Procerodes*, in experiments to test survival to hypotonic sea water that the last animals to die do so under very different conditions from those under which the first animals died, and that they lived longer because animals had previously died in the medium. This suggests

TABLE V

RELATION BETWEEN PH OF CULTURE MEDIUM AND SURVIVAL TIME OF *Paramecium*

pH of Culture	No. of Instances	Average Survival in Minutes	pH of Culture	No. of Instances	Average Survival in Minutes
6.5	4	10.5	7.4	4	15.7
6.6	.....	.....	7.5	18	15.9
6.7	4	13.5	7.6	1	11.0
6.8	5	14.0	7.7	3	17.7
6.9	4	12.5	7.8	.....	.....
7.0	2	16.0	7.9	.....	.....
7.1	10	19.9	8.0	3	9.0
7.2	11	13.8	8.1	3	9.0
7.3	11	10.0			

that there may have been a similar modification in some of the present experiments, especially those in which the survival time of very dense populations was tested. Certain of the experiments involved the survival time of more than 500 individuals in a single cc. of medium. Applying Allee's findings, it would seem almost certain that the last animals to die were present under highly modified conditions. Accordingly, a new series of experiments was set up designed to overcome this difficulty.

For the next set of experiments, cultures of 10 liters volume were used. These were seeded with approximately 0.1 animal per cc. Counts of the population were made as before. The pH readings were taken at the time of making the resistance tests. The procedure

for making the resistance test was to filter some of the medium to remove the animals, then to count back, by means of a capillary pipette, five animals into 1 cc. of the filtered medium. For a single determination of the resistance time ten to fifteen 1-cc. culture samples, each containing five animals were prepared and placed in the water bath as in the previous type of experiments. These were removed at intervals and placed on depression slides for counting. Counts were made under a binocular dissecting microscope. They were made continuously and as rapidly as possible during the time when the animals were dying. The samples were not set aside to cool, as in the preceding experiments, because it was believed that with continued practice dead animals could be distinguished from those merely rendered immobile by heat. In order to eliminate any error due to the fact that the last animals to die might live unusually long, it was decided arbitrarily to consider as the resistance time that in which only two of the animals survived. This time was established from at least three samples in each case, averages and interpolations being made when necessary. Table VI gives the result of the first of these experiments.

The population of this culture was below normal; the reproductive rate was unusually low throughout the entire period. The relationship between the pH value of the culture and the resistance time corresponds somewhat closely to that which Chalkley found, although the absolute values differ; this is due, in all probability, to differences in technique. That the difference in resistance time is not due to differences in numbers of organisms present is shown by the fact that during the last 5 or 6 days of the experiment there was little increase in the population. The culture showed an unusual variability in pH values, however, which is reflected in the resistance time.

2. *Under modified conditions.*—As a further means of testing the effect of pH, as distinguished from density of population, as the variable factor regulating the phenomenon, each day a 100 cc. sample was filtered out and acidulated with 0.1 N hydrochloric acid to pH values through which the culture had passed previously. No definite value was tried for at any time but a small amount of the dilute acid was added and the pH was tested. When a value was obtained that the culture had previously had, a small part of the sample was

set aside, and further acidulation was made. In this way two to four acidulated samples were prepared daily through most of the experiment. Then animals were washed once, and five were counted into 1-cc. amounts of each of the acidulated media. They were allowed to stand approximately 1 hour to acclimate to the changed pH and then were tested for heat resistance as in the case of the animals in the un-

TABLE VI

RESISTANCE OF *Paramecium* FROM 10-LITER CULTURE TO 40° C. WHEN EXPOSED IN GROUPS OF FIVE IN 1 CC. OF MEDIUM FROM THE CULTURE IN WHICH THEY HAD LIVED

Age of Culture	Temperature of Culture (Degrees Centigrade)	pH	Population per Cc.	Survival in Minutes
Beginning	24	6.8	0.1	.....
1 day	24	6.8	0.3	.....
2 days	24	6.8	1.5	8.5
3 days	24	6.9	7.1	9
4 days	24	7.1	19.6	8
5 days	24	7.2	30.3	7.5
6 days	24	7.5	39.7	12
7 days	24	7.6	38.6	13
8 days	24	7.7	40.1	14
9 days	24	7.7	43.6	15
10 days	24.5	7.8	45.2	16
11 days	24.5	7.9	52.0	17
12 days	24.5	7.8	59.4	16
13 days	24.7	8.0	62.2	21
14 days	25	8.0	62.8	19
15 days	25	8.1	65.1	18
16 days	25.2	8.2	66.5	15
17 days	25	8.0	66.3	19

modified medium. The results of this part of the experiment are given in Table VII. They show that resistance of animals in the later stages of a culture subjected to high temperature in their own medium which has been readjusted to the lower pH value of an earlier stage approximates that of animals that were present when the culture originally passed through that pH value.

Another experiment carried out immediately after the preceding one gave closely comparable results. These experiments indicate that under these conditions the pH of the culture exerts a greater in-

fluence on the survival time than does any direct effect of density of population.

In order to test further the effects of pH values, the density of population, and the changes in media due to having been inhabited, another experiment was performed. In the preceding experiment the water used had been Richmond, Indiana, city water, which was known to have a high calcium content. The calcium appeared to buffer the media to relatively high pH values. In order that the effects of the organisms, both bacteria and Protozoa, on the pH cycle might be, as far as possible, unhampered, a relatively unbuffered medium was sought. The medium used by Mr. Ralph B. Oesting, of the Zoölogy Department of the University of Chicago, as an artificial pond water for fish culture was found to be satisfactory, by using it in double concentration. As used in double strength, it is made by adding to each liter of distilled water, 0.4 mg. $CaCl_2$, 0.2 mg. $MgSO_4$. 17 H_2O, 0.2 mg. K_2SO_4, and 0.2 mg. $NaNO_3$. A 10-liter quantity of standard hay infusion was made, using the artificial pond water.

TABLE VII

COMPARISON OF THE TIME OF RESISTANCE OF *Paramecium* EXPOSED TO 40° C. IN UNMODIFIED AND IN REACIDULATED MEDIA FROM THEIR OWN CULTURE

Day	Ph	Population per Cubic Centimeter	Survival in Minutes in Unmodified Medium	Survival at Modified pH Values in Minutes								
				6.8	6.9	7.0	7.1	7.2	7.3	7.4	7.5	7.6
4th	7.1	19.6	8	8	....	9						
5th	7.2	30.3	7.5	9	....	8	8					
6th	7.5	39.7	12	9	....	....	....	7	9			
7th	7.6	38.6	13	8.5	9	....	....	8	....	11		
8th	7.7	40.1	14	8	....	....	....	....	10	....	13	
9th	7.7	43.6	15	....	....	8	....	....	....	10		
10th	7.8	45.2	16	8.5	....	....	....	....	....	9.5	....	15
11th	7.9	52.0	17	....	....	....	....	6	....	....	15	
12th	7.8	59.4	16	....	9	....	....	....	....	....	....	15
13th	8.0	62.2	21	....	....	....	9	....	11	....	13	
Average survival time for modified pH values				8.8	9	8.3	8.5	7	10	10.2	13.7	15

This had an initial pH of 6.2. From this, four 2-liter quantities were set aside in glass jars of approximately 4 liters capacity. These were designated as cultures "A," "B," "C," and "D." The "A" culture was left unseeded with *Paramecium* in order to check the normal pH cycle in an uninhabited culture. Cultures "B," "C," and "D" were seeded with *Paramecium* in the concentration of approximately 0.1 animal per cc. After seeding, these cultures were mixed together and reseparated into 2-liter amounts so that the initial bacterial and protozoan content might be as nearly identical as possible. The "B" culture was allowed to run through its cycle in a normal way. The "C" culture was allowed to run through its pH cycle until it came to pH 6.8. It was then held to that concentration by adding daily enough 0.1 N HCl to restore it to pH 6.8. The "D" culture was a reserve culture carried on under conditions like those of the "B" culture. Population counts were made daily from the "B" culture as described earlier in this paper. The "C" and "D" cultures had no population counts until the last day of the experiment, on account of lack of time. In maintaining the cultures the daily routine procedure was to take the pH of all the cultures, adjust the pH of the "C" culture to pH 6.8, make population counts from the "B" culture, and then run a series of resistance tests. These tests were made by using animals in groups of five as previously described. Animals were tested under various conditions as follows: animals from the "B" culture in their own filtered medium; animals from the "B" culture in medium filtered from the "C" (pH 6.8) culture; animals from the "B" culture in medium filtered from the uninhabited culture "A"; animals from the "C" culture in medium filtered from the "B" culture; and animals from the "C" culture in medium filtered from their own culture. When animals were tested in media other than their own, they were washed once in the medium into which they were being introduced, in accord with the method previously described and adopted as standard for this investigation, and then tested as quickly as possible. This procedure was adopted in an attempt to eliminate the possibility of acclimation and hereditary modifications of the sort which Jollos (1921) found to last through a number of fissions but which could not be assumed to be mutations. The data from these tests are expressed in Table VIII.

Various phases of this experiment serve to control each other, and they will be analyzed individually.

Comparison of lines (5), (6), and (8) shows that there is a marked correspondence in the pH cycles of cultures allowed to develop nor-

TABLE VIII

RESISTANCE OF *Paramecium* TO 40° C. UNDER CONTROLLED CONDITIONS OF pH, DENSITY OF POPULATION, AND CONDITION OF MEDIUM WITH RESPECT TO ITS HAVING BEEN INHABITED

"A" culture was unseeded. "B" culture was allowed to develop normally. "C" culture had pH adjusted daily to 6.8.

(1) Day of experiment	1	2	3	4	5	6	7	8
(2) Population of "B" culture per cc	0.1	.....	1.6	10.2	49.7	261.0	354.7	360.0
(3) Population of "C" culture per cc	0.1	.....	.....	.....	.....	.....	.....	428.6
(4) Population of "D" culture per cc	0.1	.....	.....	.....	.....	.....	.....	313.7
(5) pH of "A" culture	5.7	6.8	7.0	7.2	7.25	7.3	7.4	7.4
(6) pH of "B" culture	5.7	6.7	7.0	7.2	7.25	7.3	7.45	7.7
(7) pH of "C" culture: Before adjustment	5.7	6.8	7.1	6.9	7.0	7.0	7.0	6.9
After adjustment	.....	.....	6.8	6.8	6.8	6.8	6.8	6.8
(8) pH of "D" culture	5.7	6.8	7.1	7.2	7.25	7.35	7.4	7.4
(9) Resistance of "B" animals in own medium	.....	.....	13	13.5	14	14.5	10	{*20 18
(10) Resistance of "B" animals in "A" medium	.....	.....	13	14.5	16	19	11	{*23 18
(11) Resistance of "B" animals in "C" medium	.....	.....	10	12	12	13	10	*11
(12) Resistance of "C" animals in own medium	.....	.....	13	13.5	12	11	10	15
(13) Resistance of "C" animals in "B" medium	.....	.....	13	13	11	9.5	10	†11

* Indicates substitution of animals from "D," another culture comparable to "B," because conjugation was observed in "B."

† Indicates substitution of medium from culture "D" for that of "B."

mally. An exception to this is to be seen in the 8th-day pH value in line (6). On the 7th day a number of conjugating pairs of animals were seen in the "B" culture. No suggestion is offered by way of correlating these two facts. The daily drive toward neutrality in the "C" culture which was readjusted daily to pH 6.8 was quite con-

stant in its magnitude; the value was either 0.1 or 0.2 pH points in every case.

A slight advantage to the animals in holding the pH at the constant figure of 6.8 over allowing it to develop normally is suggested, as is seen by comparing line (3) with (2) and (4). It was noted during the last 2 days of the experiment that the animals in the "C" culture appeared more plump and more active. This suggests, at least in certain aspects, that the high pH characteristic of old cultures is relatively detrimental to the animals populating them.

With respect to the time of resistance to high temperature a number of generalizations can be made. By comparing (9) and (10) it is seen that under the conditions of sudden exposure to high temperature previously uninhabited medium of an equivalent pH tends to be more satisfactory for the animals than their own medium which has been affected by their own presence. In applying "Student's" formula for testing the statistical significance of small numbers of paired experiments to the figures in lines (9) and (10), there is obtained a probability of 0.0466, which approaches statistical significance. By comparing the successive resistance times as shown in lines (9) and (10), it is seen that there is a tendency for the resistance times to increase day by day. This is correlated with two variables—the increase in population and the increase in pH value. However, since the animals live fully as long or longer in uninhabited medium than in that which has contained these organisms, it appears again that pH is the more significant factor. It should be recalled that the seventh-day readings in lines (9), (10), and (11) involve animals which were undergoing conjugation. It is not known what the cause-and-effect relationships are here; however, the decided drop from the previous day in each case implies a changed physiological state in connection with conjugation. That the phenomenon is a real one and not due to the accident of selecting abnormal animals for the test is indicated by the fact that these parts of the experiment were repeated twice immediately after the initial test, with identical results. The resistance of animals kept constantly at or near pH 6.8 does not undergo the same type of daily increase as does that in the cases where the pH tends to rise. The reading for the last day, however, for the animals kept constantly at pH 6.8 and tested in their

own media (line 12) is not easily explained on these grounds. Another part of the experiment which does not bear out the hypothesis that the important factor is pH is that in which the animals from the constant pH 6.8 culture were tested in medium from the inhabited culture undergoing normal pH changes (line 13). It should be borne in mind, however, that the tests were made as soon as possible after the counting-out process was completed, so that the results may express the condition of the animals rather than the condition of the culture.

To summarize the results of this experiment: The animals tend to increase their resistance to sudden exposure to heat with the increase in population and the rise in pH. Animals kept at a constant pH value tend to maintain a more nearly constant resistance time to 40° C., despite the fact that the population is rapidly increasing. For animals living in normally changing medium with respect to pH, there are indications that uninhabited medium of equivalent pH results in greater resistance time than their own medium, indicating that the changes produced by the animals themselves do not necessarily have autoprotective results and, in fact, may be harmful. Following this reasoning, then, it appears that the pH changes in a culture alone may account for the fact that densely populated cultures are more resistant to heat than new and isolation cultures.

DISCUSSION

The problem of the resistance of Protozoa to high temperature is not a simple one. Perhaps no other environmental condition than heat has more accompanying variable factors. In the use of depression-slide cultures at temperatures which are lethal only after some hours, the culture medium becomes radically changed from the initial condition. Evaporation of the medium takes place even in almost saturated atmospheres, at times until the culture has only a fraction of its original volume. This implies, of course, a corresponding concentration of the substances dissolved in the medium. Since Chalkley has shown that excesses of some salts affect the death time of Paramecia under high temperature conditions, evaporation must, at times, have marked consequences. Also, high temperature tends to drive out the dissolved gases, giving another type of radical

change. Heat greatly accelerates bacterial growth, and ever so slight initial differences in bacterial content of cultures become highly magnified with the passage of time. Correlated with bacterial growth, pH changes are rapid and drastic in small cultures. Jennings (1913) and others have called attention to the fact that the existence of Protozoa on depression slides is precarious.

On account of these difficulties, the depression-slide method of testing the heat resistance at barely lethal temperatures, of Paramecia is felt to be unreliable. The use of mass cultures eliminates, to a degree, the differences in environmental conditions of the organisms.

The temperature of 40° C. was selected for the mass-culture experiments because of the short time required for making the survival tests, thus doing away with many confusing changes in the medium. Experiments showed that animals which die in 15 minutes at 40° C. may live 1½ hours at 38° C., and some will live for as much as 6 days at 34° C. if mass cultures are used. The question arises at once as to whether a high degree of resistance to death at 40° C. where the death time is a matter of some 10–20 minutes is correlated with resistance at a lower temperature of, say, 34° C., at which temperature, as has just been stated, the same animals would live about 6 days. The data in this investigation do not answer this question directly, and further research is needed to determine the extent of this correlation, if any exists. However, the differences in death time at 40° C. imply variations in the physiological states in the animals under various conditions.

Robertson's original observations on the advantage of mass cultures over isolation cultures for the survival of Protozoa in high temperatures were made in his laboratory in the course of the hot season of the year. The probability is that, on the whole, the temperature rose gradually. This raises the question whether from the large numbers of individuals in mass cultures some might have developed an acclimatization to the changed condition while others perished, and whether the race might have become more resistant through the reproduction of these selected individuals. The inheritance of acclimatization characteristics has been reported frequently for Protozoa. Dallinger (1887; cited by Jennings, 1929) cultivated three

species of flagellates under conditions of very gradually increasing temperatures until the organisms were able to thrive at a temperature of 70° C. Ritz (1916) found that, as antibodies of the host act on trypanosomes, most of the organisms are killed, but that those that survive develop a resistant strain. However, Jollos (1921) practiced selection of resistant individuals of *Paramecium* to heat but found no hereditary diversities within a single clone. This coincides with Jennings' findings (1929) that within a single clone the animals are remarkably constant in their hereditary characteristics. If it can be assumed that Robertson's organisms were from a single clone, the possibility of hereditary acclimation characteristics would seem scarcely sufficient to account for his observed phenomenon.

Another possible source of difference in response in Protozoa when the small numbers of isolation and small group cultures are studied is the difference in time since the last fission. Some observations based on very few instances have indicated that animals in fission are more than usually resistant to sudden increase in temperature, while conjugation pairs are highly susceptible to heat. From this one would infer, as might be expected, that there are changes in physiological states during the interfission and interconjugation periods.

No attempt was made to study the various effects of different species of bacteria in the cultures at any given time or of bacterial succession. It is possible that bacteria play an important rôle in the phenomenon under consideration; the investigation of this question is, however, reserved for the present.

Since the data in this investigation indicate that the pH of the culture medium plays a large part in determining the resistance to high temperature of *Paramecium*, the question should be considered whether the animals aid in bringing about the pH changes. It has been noted that the usual course in a culture is toward an increased pH value while the numbers of animals are increasing. This increase in pH value frequently results in increasing the resistance of the animals to heat. If it can be shown, as Darby suggested, that the animals actually do aid in adjusting the pH to an optimum, a case would be made for group protection of a sort implied in Robertson's extension of his theory of allelocatalysis. However, the experiments

here cited indicate that bacterial cultures run their pH cycle in a practically identical manner, regardless of whether *Paramecium* are present. Chalkley found that pH values above 8.0 have a harmful effect in that they shorten the resistance time of *Paramecium* to high temperatures. In these experiments this same tendency was indicated. Losina-Losinsky (1931) stated that Paramecia rapidly lose the ability to ingest bacteria as the pH increases above 8.0. In many cases the *Paramecium* cultures reach a pH stability in this high range.

In order to see whether some other factor than the presence of *Paramecium* in cultures affected the pH, a series of depression-slide cultures of various sizes were made, using freshly boiled and cooled,

TABLE IX

EFFECT OF SIZE OF CULTURE ON RATE OF PH CYCLE

Cultures are made from a 10-liter volume of freshly boiled and cooled, bacterized medium. The water is Oesting's artificial pond water.

No. of Cultures for Each Reading	Size of Culture	pH at Beginning	pH at 16 Hours	pH at 24 Hours
32	0.1 cc.	6.2	6.8	7.5
20	0.25 cc.	6.2	6.6	7.5
10	1.0 cc.	6.2	6.1	7.3
1	10 liters	6.2	5.7	5.7

bacterized standard hay infusion. These were made in the following numbers and sizes: sixty-four 0.1-cc. cultures in small depression slides; forty 0.25-cc. cultures in depression slides; twenty 1-cc. cultures in large depression slides of 2 cc. capacity each. The original 10-liter volume of infusion served as a control. The composite pH of half the cultures of each size group was taken at the end of a 16-hour period. The composite pH of the other half of each size group was taken at the end of 24 hours. Table IX summarizes these readings. These data show a marked inverse correlation between the size of the culture and the rate of the pH cycle.

Recalling Robertson's observations which motivated this investigation—namely, that single individuals die when isolated from stock cultures under conditions of the high temperatures found at times in his laboratory, while the stock cultures continue to live—this ob-

servation has been verified and the following explanations are suggested:

If the cultures used by Robertson did not arise from a single infusion, variations might be expected which would allow a greater number of heat-resistant forms to be found in the mass cultures than among the relatively few isolated animals. If Robertson's animals did come from a single clone, variations in physiological states such as accompany the fission cycle might favor the survival of animals in the mass cultures, or some of them, as compared with the fewer number isolated. But entirely aside from these possibilities, an explanation may be found in the relation between pH of the medium and resistance to heat—at least in the case of *Paramecium caudatum*.

An isolation culture is usually made by taking an animal from a stock culture of some considerable size and placing it in a depression slide with a small amount of fresh medium. The pH in these small cultures is rapidly carried into a range which decreases the resistance of the animal to heat. Chalkley (1930) pointed out three ranges of low resistance: below pH 6.7, around 7.2, and above 8.2. On the other hand, the stock culture has a retarded pH cycle which moves slowly through the range of greatest resistance—pH 7.4 to 8.0. Thus, pH effects alone seem sufficient to account for the observed phenomenon without the aid of Robertson's extension of his allelocatalytic hypothesis.

SUMMARY

1. Robertson (1924*b*), using *Enchelys farcimen*, observed that mass cultures withstand high temperatures better than do isolation cultures. He postulated an "allelocatalytic" substance given off by the animals which serves for mutual protection. This phenomenon has been re-examined and verified under certain conditions, without, however, verifying Robertson's explanation.

2. The isolation-culture method of testing the resistance of Protozoa to high temperatures was found to be impracticable because of the difficulty of controlling, at the temperatures used, evaporation rate, salt, dissolved gas and bacterial content, and pH value of small cultures.

3. A mass-culture method was developed; the cultures were held at room temperatures and sample individuals were taken out and tested under various conditions.

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4. After experimentation, a temperature of 40° C. was adopted as a testing temperature for time of resistance of the organisms because it usually brought about death in from 10 to 20 minutes and thereby eliminated uncontrollable factors inherent in tests requiring much longer time.

5. The general conditions of greatest resistance to a sudden exposure to a temperature of 40° C. were those furnished by mass cultures which had become thickly populated and had reached a relatively high pH value. The relative importance of density of population and pH of the culture medium was studied, and it was found that pH is the more important in determining resistance time.

6. Paramecia do not affect materially the pH cycles of cultures.

7. In the normal course of pH cycles, cultures pass through two or three of the pH ranges where the resistance of the animals to heat is low. These ranges lie around the pH values of 5.7, 7.2, and 8.3, with ranges of greater resistance between, i.e., around pH 6.8 and 7.8 (cf. Chalkley, 1930). Chalkley reports that these ranges may be modified by excess amounts of certain salts.

8. The rate of change of pH of bacterized cultures within the limits tested is inversely proportional to the size of the culture. The difference in rate between depression-slide and 10-liter mass cultures is recorded.

9. It is recognized that a possible factor in the greater resistance to high temperatures of Paramecia from mass cultures is the greater number of animals present, with a correspondingly greater diversity of resistance abilities; and that the survival may be due to selection among these diverse forms.

10. It is suggested that the pH relationship alone seems sufficient to account for the observed phenomenon of the greater resistance to high temperatures with mass cultures than with isolation cultures among Protozoa, without the aid of Robertson's extension of his allelocatalytic hypothesis.

LITERATURE CITED

ALLEE, W. C. 1931. Animal aggregations: A study in general sociology. Chicago: University of Chicago Press. Pp. 431.

———. 1934. Recent studies in mass physiology. Biol. Rev., **8**:1–48.

ALLEE, W. C., and BOWEN, EDITH S. 1932. Studies in animal aggregations: mass protection against colloidal silver among goldfishes. Jour. Exp. Zoöl., **61**:185–207.

BROWN, A. M., and BANTA, L. A. 1932. Sex control in Cladocera. VII. Male production in relation to temperature. Physiol. Zoöl., **5**:218–29.

CARPENTER, KATHLEEN E. 1927. The lethal action of soluble metallic salts on fishes. Brit. Jour. Exp. Biol. **4**:378–90.

CHALKLEY, H. W. 1930. On the relation between the resistance to heat and the mechanism of death in *Paramecium*. Physiol. Zoöl., **3**:425–40.

CHILD, C. M. 1915. Senescence and rejuvenescence. Chicago: University of Chicago Press. Pp. 481.

DALLINGER, W. H. 1887. The President's address. Jour. Roy. Micr. Soc. **1**: 185–99.

DARBY, HUGH H. 1930. Studies on growth acceleration and yeast. Jour. Exp. Biol., **7**:308–15.

DRZEWINA, A., and BOHN, G. 1926. Influence de la température sur le sperme d'Oursin, en fonction de la masse. Compt. Rend. Soc. de Biol., **95**:376–78.

———. 1928. Les Convoluta. Ann. Sci. Nat. Zool., X Ser., **11**:299–398.

HEILBRUNN, L. V. 1924. The colloid chemistry of protoplasm. III. The viscosity of protoplasm at various temperatures. Amer. Jour. Physiol., **68**:645–48.

HUTCHISON, R. H. 1913. Some specific differences among Protozoa with respect to their resistance to heat. Jour. Exp. Zoöl., **15**:131–44.

———. 1915. The effects of certain salts and of adaptations to high temperatures on the heat resistance of *Paramecium caudatum*. *Ibid.*, **19**:211–24.

JAHN, T. L. 1928. Relation of volume of culture medium to growth rate of *Euglena*. Anat. Rec., **41**:54.

———. 1929. Studies on the physiology of the euglenoid flagellates. I. The relation of the density of population to the growth rate of *Euglena*. Biol. Bull., **57**:81–106.

JENNINGS, H. S. 1913. The effect of conjugation in *Paramecium*. Jour. Exp. Zoöl., **14**:279–391.

———. 1929. Genetics of the Protozoa. Bibliographia Genetica, **5**:106–330.

JOHNSON, W. H. 1933. Effects of population density on the rate of reproduction in *Oxytricha*. Physiol. Zoöl., **6**:22–54.

JOLLOS, V. 1921. Experimentelle Protistenstudien. I. Untersuchungen über Variabilität und Vererbung bei Infusorien. Arch. f. Protistenk., **43**:1–222.

JONES, EDGAR P. 1930. Hydrogen-ion changes in hay and hay-flour infusions. Biol. Bull., **59**:275–84.

KALMUS, H. 1929. Versuche über die Teilung von *Paramecium caudatum* in der Kapillare nebst Bemerkungen über den sogennanten Raumfaktor. Arch. f. Protistenk., **64**:402–8.

———. 1931. *Paramecium*, das Pantoffeltierchen. Jena: Gustav Fischer. Pp. 188.

LOSINA-LOSINSKY, L. K. 1931. Zur Ernährungsphysiologie der Infusorien. Arch. f. Protistenk., **74**:18–120.

MYERS, E. C. 1927. Relation of density of population and certain other factors to survival and reproduction in different biotypes of *Paramecium caudatum*. Jour. Exp. Zoöl., **49**:1–43.

PLOUGH, H. H. 1917. The effect of temperature on crossing over in *Drosophila*. *Ibid.*, **24**:147–209.

RITZ, H. 1916. Über Rezidive bei experimenteller Trypanosomiasis. II. Mitteilung. Arch. f. Schiffs- und Tropenhyg., **20**:392–420.

ROBERTSON, T. B. 1921*a*. Experimental studies on cellular multiplication. I. The multiplication of isolated Infusoria. Biochem. Jour., **15**:598–611.

———. 1921*b*. Experimental studies on cellular multiplication. II. The influence of mutual contiguity upon reproductive rate and the part played therein by the X-substance in bacterized infusions which stimulates the multiplication of Infusoria. *Ibid.*, pp. 612–19.

———. 1924*a*. The influence of washing upon the multiplication of isolated Infusoria and upon the allelocatalytic effect. Austral. Jour. of Exp. Biol. and Med. Sci., **1**:151–75.

———. 1924*b*. Allelocatalytic effect in cultures of *Colpidium* in hay infusion and in synthetic media. Biochem. Jour., **18**:1240–47.

"Student." 1925. New tables for testing the significance of observations. Metron, **5**:18–21. Tables 26–32.

THE ABILITY OF FRESH-WATER FISH TO EXTRACT OXYGEN AT DIFFERENT HYDROGEN-ION CONCENTRATIONS[1]

(Three figures)

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POWERS (1921) concluded that the ability of marine fishes to absorb oxygen at low tension from sea water is more or less dependent upon the hydrogen-ion concentration of the water. The method used by Powers was briefly as follows: Fish were placed in jars of a suitable size filled with sea water. After that the jars were sealed with rubber stoppers. After all the movements of the fish had ceased the pH and the oxygen were determined immediately. The amount of oxygen left at death was taken to represent the minimum concentration below which the fish was unable to extract any more oxygen from the water at that hydrogen-ion concentration. Powers found that the cunner (*Tautogolabrus adspersus* Walbaum) was able to extract oxygen from the sea water down to approximately 0.25 cc. per liter (0.36 p.p.m.) before asphyxiation occurred at all hydrogen-ion concentrations up to 6.5 (6.5–8.8). The butter fish (*Poronotus triacanthus* Park) also showed very little difference between pH 7.0–9.0. However, in the case of the herring (*Culpea harangus L.*) there was a very abrupt change in the ability of this species to utilize oxygen at low tension under different hydrogen-ion concentrations.

Pruthi (1926) has criticized the work of Powers because the latter did not determine the initial oxygen. Pruthi worked with the stickleback (*Gasterosteus aculeatus*). He modified Powers' procedure by determining the pH and the oxygen at the beginning of the experiment as well as at the end. On the basis of his own work Pruthi concluded that the amount of oxygen present at death at any pH depends on the amount of oxygen present in the water at the beginning of the experiment. To quote from Pruthi:

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The most crucial test by which Powers could have tested his results and their interpretation was to observe on a fresh fish the effect of the water in which one had died previously, presumably on account of the fact that it could not absorb O_2 at that pressure; i.e., for instance, if the herring, *Clupea*, was observed to die at pH 7.1, leaving 2.5 cc. per litre concentration, Powers should have put another herring in the same water to see whether the fish died in a short time, which it should have done if this view was correct! Powers did not test his conclusion in this manner.

Pruthi applied this test to his experiments with sticklebacks. His procedure was as follows: Fish were introduced into jars containing water of known pH and oxygen concentrations. The jars were sealed and the time required for the fish to die was determined. After one fish had died it was removed and another one substituted. As many as six lots were introduced into some jars in this way, and it was found that the fish of the last lot survived almost as long as did the fish of the first lot. Modifying his procedure slightly from the above, Pruthi found that sticklebacks introduced into water of pH 3.0 and oxygen 1.36 cc. per liter survived just as long as stickleback introduced in water of the same pH but with an initial oxygen of 6.5 cc. per liter. By still another series of experiments he showed that over the pH range 2.4–3.7 the oxygen concentration at the time when the fish die depends on the initial concentration of oxygen.

The purpose of the present paper is to report the results of a number of experiments performed to test the conclusions of Pruthi and Powers in so far as they apply to several species of fresh-water fish. The validity of their conclusions will be discussed in connection with our own data. The method followed here is Pruthi's modification of Powers' method, i.e., the pH and the oxygen were determined at the beginning of the experiment (initial oxygen) as well as at the end (lethal oxygen). The oxygen was determined by the Winkler method. The pH values above 7.0 were determined colorimetrically, those below 7.0 with the quinhydrone electrode.

The fish used in these experiments are as follows[2]: the nine-spined stickleback (*Eucalia inconstans* Kirtland), largemouth black bass (*Huro floridana* Le Sueur), smallmouth blackbass (*Micropterus do-*

[2] The nomenclature used is taken from a check-list of the fishes and fishlike vertebrates of North and Middle America, north of the northern boundary of Venezuela and Colombia, by Jordan, Everman, and Clark.

lomieu Lacepede), bluegill (*Helioperca incisor* Cuv. and V.), golden shiner (*Notemigonus crysoleucas* Mitchill), the green sunfish (*Apomotis cyanellus* Rafinesque), the goldfish (*Carassius auratus L.*), the bluntnosed minnow (*Hyborhynchus notatus* Rafinesque), the white crappie (*Pomoxis annularis* Rafinesque), the black crappie (*Pomoxis sparoides* Lacepede), the orange spotted sunfish (*Allotis humilis* Girard), the rainbow trout (*Salmo shasta* Jordan), the yellow perch (*Perca flavescens* Mitchill), and the steel-colored minnow (*Erogala whipplii* Girard).

The size of fish used.—Preliminary tests showed that variations in size from 16 grams to 36 grams produced no appreciable effect upon the lethal oxygen concentration, i.e., a fish that weighed 16 grams died at the same oxygen concentration as did a fish weighing 36 grams under the same experimental condition. The same holds true for fish ranging in weight from 5.2 to 7.3 grams. There is, however, a difference in the lethal oxygen in a fish of weight group 5.2–7.3 grams and weight group 29.5–42.5, for instance. This latter condition is especially noticeable under unfavorable conditions of hydrogen-ion concentration. The larger fish leave a smaller amount of oxygen in the water that is either extremely acid or extremely alkaline.

DATA AND DISCUSSION

I. EFFECT OF INITIAL OXYGEN UPON LETHAL OXYGEN

In Table I there are recorded some typical results that show the effect of the initial oxygen upon the lethal oxygen at different hydrogen-ion concentrations. Experiments, 2 3, 4, and 7 of Table I show that the initial oxygen has very little effect upon the lethal oxygen in the case of the bass (*Huro floridana*) around pH 7.05–7.2 and at pH 9.1. In Experiments 1 and 8 the bass with the high initial oxygen died at a lower oxygen concentration than those with the lower initial oxygen at the same pH and temperature. This result is contrary to Pruthi's conclusion. Experiments 5, 6, 9, and 10 (Table I) show that at pH 3.0–3.8 and again at pH 9.7 and 10.75 the bass with the higher initial oxygen die at a higher lethal oxygen than those with lower initial oxygen. Experiments 11 and 12 show that the bluntnosed minnows (*Hyborhynchus notatus*) at pH 6.95 and 6.55 behave contrary to Pruthi's prediction, i.e., the fish with the

TABLE I

THE EFFECT OF INITIAL CONCENTRATION OF DISSOLVED OXYGEN UPON THE LETHAL CONCENTRATION

(Values for oxygen are expressed in parts per million. Values for hydrogen-ion concentration are expressed in pH units.)

Species	No. of Exp.	No. of Fish Used	Initial O_2	Lethal O_2	pH	Temp. ° C.	Wt. in Grams
Largemouth Blackbass (*Huro floridnana*)	1	6	5.6	0.65	6.95	18.0	5.2 – 7.3
	1	6	8.0	0.45	6.95	18.0	5.5 – 7.8
	2	6	3.7	0.79	7.05	25.0	29.5 –42.5
	2	6	8.0	0.80	7.05	25.0	16.0 –36.0
	3	3	3.1	0.72	7.2	15.5	19.0 –24.0
	3	3	8.2	0.70	7.2	15.5	24.0 –32.5
	4	3	4.58	0.27	7.1	13.5	17.0 –17.0
	4	3	10.50	0.25	7.1	13.5	17.0 –18.0
	5	3	5.2	4.8	3.0	19.0	5.3 – 7.1
	5	3	8.4	8.0	3.0	19.0	3.6 – 4.9
	6	3	5.2	4.7	3.8	19.0	4.3 – 5.6
	6	3	8.4	7.6	3.8	19.0	2.6 – 3.4
	7	3	8.0	0.47	9.1	16.5	26.0 –34.0
	7	3	13.5	0.50	9.1	16.5	32.0 –38.0
	8	3	6.97	0.50	6.15	15.0	19.0 –21.0
	8	3	11.64	0.33	6.20	15.0	17 –27
	9	1	6.3	3.0	9.7	20.0	5.2
	9	1	8.4	4.9	9.7	20.0	4.47
	10	1	6.3	5.7	10.75	20.0	4.85
	10	1	8.4	8.1	10.75	20.0	5.64
Bluntnose Minnow (*Hyborhynchus notatus*)	11	6	1.8	1.08	6.95	16.0	3.7 – 4.8
	11	6	3.6	1.02	6.95	16.0	3.4 – 4.5
	12	3	2.6	1.20	6.55	16.0	3.6 – 3.9
	12	3	5.1	0.95	6.55	16.0	2.8 3.9
	12	2	5.0	4.5	10.5	20.0	3.6 – 3.9
	12	2	8.2	7.0	10.0	20.0	3.8 – 5.5
White Crappie (*Pomoxis annularis*)	13	3	4.58	0.22	7.1	13.5	8.0
	13	3	10.50	0.23	7.1	13.5	7.0
Bluegill (*Helioperca incisor*)	14	3	4.58	0.31	7.1	13.5	9.0 –10.0
	14	3	10.50	0.30	7.1	13.5	7.0 – 8.0
Green Sunfish (*Apomotis cyanellus*)	15	3	4.58	0.31	7.1	13.5	3.0 – 5.0
	15	3	10.50	0.31	7.1	13.5	3.0 – 4.0
	15	1	6.3	1.70	9.6	20.0	10.3
	15	2	8.4	2.35	9.6	20.0	7.87
	15	1	6.3	4.9	10.75	20.0	14.65
	15	1	8.5	6.7	10.75	20.0	11.29
	15	1	8.2	6.1	10.7	.....	.....
Golden Shiner (*Notemigonus Crysoleucas*)	16	3	4.58	0.16	7.1	13.5	33.0 –42.0
	16	3	10.50	0.14	7.1	13.5	32.0 –37.0
Yellow Perch (*Perca flavescens*)	17	3	4.58	0.35	7.1	13.5	32.0 –36.0
	17	3	10.50	0.35	7.1	13.5	37.0 –64

higher initial oxygen die at a lower concentration than those fish of the same species with a lower initial oxygen. Experiments 13, 14, 15, 16, and 17 show that at pH 7.1 the initial concentration of oxygen has very little if any effect upon the lethal concentration of oxygen in the case of crappie (*Pomoxis sparoides*), bluegill (*Helioperca incisor*), green sunfish (*Apomotis cyanellus*), golden shiner (*Notemigonus crysoleucas*), and yellow perch (*Perca flavescens*). Experiment 15 shows that at pH 9.6 and pH 10.75 the initial oxygen has a very marked effect upon the lethal oxygen in the case of the green

TABLE II

RESULTS WHEN FISH WERE ADDED INDIVIDUALLY, AND REPLACED BY A LIVE MINNOW AS SOON AS DEAD
(Temperature 18° C., Oxygen 9.0 p.p.m., and pH 3.1.)

Flask 1 B			Flask 2 B		
No. of Fish	Wt. Grams	Min. Survived	No. of Fish	Wt. Grams	Min. Survived
1	0.84	31	1	1.1	29
2	0.71	31	2	1.36	32
3	0.78	22	3	0.71	27
4	0.71	23	4	0.64	22
5	0.78	19	5	0.78	20
6	0.78	33	6	0.78	23
7	1.19	33	Final O_2=6.8 p.p.m.		
8	1.19	32	Final pH=3.3		
Final O_2=6.2 p.p.m.					
Final pH=3.3					

sunfish (*Apomotis cyanellus*). It appears, therefore, that within the normal pH range to which these species of fish are acclimated, the initial oxygen has either no effect upon the lethal oxygen or the effect is in a direction opposite from that predicted by Pruthi.

In Table II are shown the results of an experiment with sticklebacks. Starting with water of pH 3.1 and an oxygen concentration of 9.0 p.p.m. a stickleback was placed in a flask and the time required for the fish to die was determined. After the fish had died it was removed and another fish substituted. In this way eight fish were used in one flask and six in another. As the table shows, successive sticklebacks required about the same length of time to die. After

eight fish had been killed in flask 1 *B* and six in 2 *B* the dissolved oxygen still amounted to 6.2 and 6.8 p.p.m., respectively.

The results obtained in the preceding paragraph cannot be duplicated within the normal pH range. At pH 7.3, 7.8, and 8.2 sticklebacks of the same size as used above died when the oxygen was down to 0.30 p.p.m. (initial oxygen 9.0 p.p.m.) and the survival time ranged from 702 minutes to 725 minutes in the same volume of water as used above. At pH 5.7 sticklebacks died when the oxygen equaled 0.80 p.p.m. When new fish were introduced into the water in which fish had died at oxygen 0.30 p.p.m. or at 0.80 p.p.m., these new fish died in a few minutes, showing thereby that at these pH values these fish could not extract oxygen from the water below 0.30 and 0.80 p.p.m., respectively. In fact these fish died for want of oxygen, whereas those in the preceding paragraph were killed not by asphyxiation caused by a lack of oxygen in the medium, but because of a paralysis of the respiratory mechanism due to the abnormally high hydrogen-ion concentration.

In another experiment with sticklebacks five fish were placed in each of two flasks containing water of pH 3.1 and an oxygen content of 9.0. One of the flasks was then sealed with a rubber stopper and the other one was attached to a tank of compressed oxygen and the latter was permitted to bubble through the flask. The sticklebacks in the sealed flask were all dead in 25 minutes when the oxygen was down to 8.6 p.p.m. The sticklebacks in the second flask were all dead in 26 minutes when the oxygen amounted to 15.6; it had been raised to this figure from 9.0 by bubbling oxygen through it. This experiment would seem to show that these fish could not live at this pH (3.1) no matter what the oxygen concentration was.

The fallacy of Pruthi's criticism lies in the fact that he tested Powers' results obtained largely within the normal range of pH by his own results, which were obtained in an abnormally low pH or abnormally high hydrogen-ion concentration.

In Table III there are represented some miscellaneous experiments showing (1) the effect of abnormally high hydrogen-ion concentration upon the lethal oxygen concentration, and (2) the lethal oxygen for some fish considerably larger than those shown in Table I or in the graphs below.

TABLE III

DATA ON VARIOUS MISCELLANEOUS FISHES SHOWING INITIAL OXYGEN AND LETHAL OXYGEN AT VARIOUS HYDROGEN-ION CONCENTRATIONS

(The fish used here are in some instances considerably larger than the fish used in Table I or in the graphs in Section III.)

Species	No. of Fish Used	Initial O_2	Lethal O_2	pH	Temp. ° C.	Wt. in Grams
Largemouth Blackbass (*Huro floridana*)	2	8.2	5.1	10.0	20.0	7.8– 9.4
	2	8.2	5.9	10.0	20.0	17.4–18.0
	6	5.2	0.43	8.3	15.0	4.4– 6.0
	6	5.0	0.53	6.3	17.0	4.5– 7.4
	5	5.0	0.42	6.25	17.0	23.4–48.5
	1	5.0	4.7	10.6	20.0	3.4
	1	5.0	3.6	10.5	20.0	3.4
Green sunfish (*Apomotis cyanellus*)	1	5.0	3.8	10.7	20.0	4.0
	1	5.0	2.2	10.4	20.0	5.0
	2	5.2	0.36	7.7	16.5	29.5–36.8
	2	5.3	0.40	8.75	16.5	44.9–45.5
	1	5.0	0.30	6.25	17.0	29.3
Orange spotted sunfish (*Allotis humilis*)	1	5.0	3.7	10.6	20.0	8.4
	1	5.0	3.0	10.4	20.0	14.0
	1	5.2	0.50	7.7	16.5	23.6 The same fish
	1	5.0	0.50	6.25	17.0	23.4 The same fish
Golden Shiner (*Notemigonus Crysoleucas*)	1	5.0	4.0	10.3	20.0	6.7
	1	5.0	4.1	10.5	20.0	6.5
	2	5.0	0.17	6.28	17.0	11.2–21.3
Black Crappie (*Pomoxis sparoides*)	1	5.0	0.40	6.25	17.0	25.4 The same fish
	1	5.3	0.60	9.0	16.5	24.0 The same fish
White Crappie (*Pomoxis annularis*)	1	5.3	0.70	9.0	16.5	12.2
	1	5.0	0.60	6.3	17.0	19.5
Bluegill (*Helioperca incisor*)	2	5.0	3.1	10.45	20.0	4.0– 5.0
	2	8.2	5.7	10.0	20.0	3.7– 4.2
	3	5.2	0.36	8.2	15.6	4.0– 4.7
	2	5.3	0.70	9.05	16.5	16.3–23.6
	2	5.3	0.70	8.9	16.5	9.3– 9.5
	1	5.0	0.40	6.25	17.0	22.3
	1	8.5	7.6	10.75	20.0	11.1
	3	5.2	0.36	7.7	16.5	17.7–22.8
Goldfish (*Carassius auratus*)	3	5.2	0.13	7.25	16.5	28.5–30.8
	1	6.3	2.80	10.7	20.0	22.9
	1	6.3	0.20	9.65	20.0	21.6
	1	5.3	0.20	7.1	16.5	54.0
	1	5.3	0.25	8.5	16.5	22.0

Of special interest here are the last two results for the orange spotted sunfish and the black crappie, as in either case the same individual was used in two experiments. In the first experiment the fish were left in the experimental flask until all movements had ceased; then they were taken out carefully and placed in water of high oxygen concentration (30–35 p.p.m.) where they revived. After several days they were used for a similar experiment.

Table III shows that the same orange spot died at the same oxygen at pH 7.7 and 6.25, namely, at 0.50 p.p.m. of oxygen. The crappie died at 0.60 p.p.m. oxygen at pH 9.0 and at 0.40 p.p.m. of oxygen at pH 6.25.

II. EFFECT OF pH ON THE ABILITY OF FISH TO EXTRACT OXYGEN FROM THE WATER AT LOW OXYGEN TENSION

Notemigonus crysoleucas Mitchill (adults).—Figure 2 shows that this species could use the oxygen down to approximately 0.2 p.p.m. from pH 6.3 to 8.1. Even at pH 5.6 the lethal oxygen was only 0.25 p.p.m., and at pH 5.1 it was 0.6 p.p.m. At pH 9.0 the lethal oxygen was considerably higher than at 8.1. On the whole this species exhibits a marked tolerance to differences in pH.

Apomotis cyanellus Rafinesque (fingerlings 2.0–2.5 inches).—This species seems best able to extract oxygen from the water at pH 6.9–8.1. But on the whole there is very little difference in the ability of this species to use oxygen at low pressure over the range of 6.4–9.2. At pH values less than 6.4 the ability of this species to use oxygen decreases very rapidly (Fig. 2).

Helioperca incisor Cuv. and V. (2-inch fingerlings).—This species has apparently a narrower pH range at which it can extract oxygen from the water at low oxygen tension than several of the species studied. The optimum pH range probably extends from approximately pH 7.0–8.5. Beyond this range in either direction there is a marked increase in the oxygen present when the fish die (Fig. 1).

Micropterus dolomieu Lacepede (2.5-inch fingerlings).—This species (Fig. 2) exhibits a tolerance to wide differences in hydrogen-ion concentration. While the lowest value for lethal oxygen seems to occur at pH 6.9 and 7.5, there is very little difference in the lethal oxygen over the entire pH range from 6.2 to 9.6. The minimum

oxygen tension at which this species can extract oxygen from the water at 25° C. appears to be about 0.4 p.p.m. At pH 3.2 or 10.7 this

Fig. 1

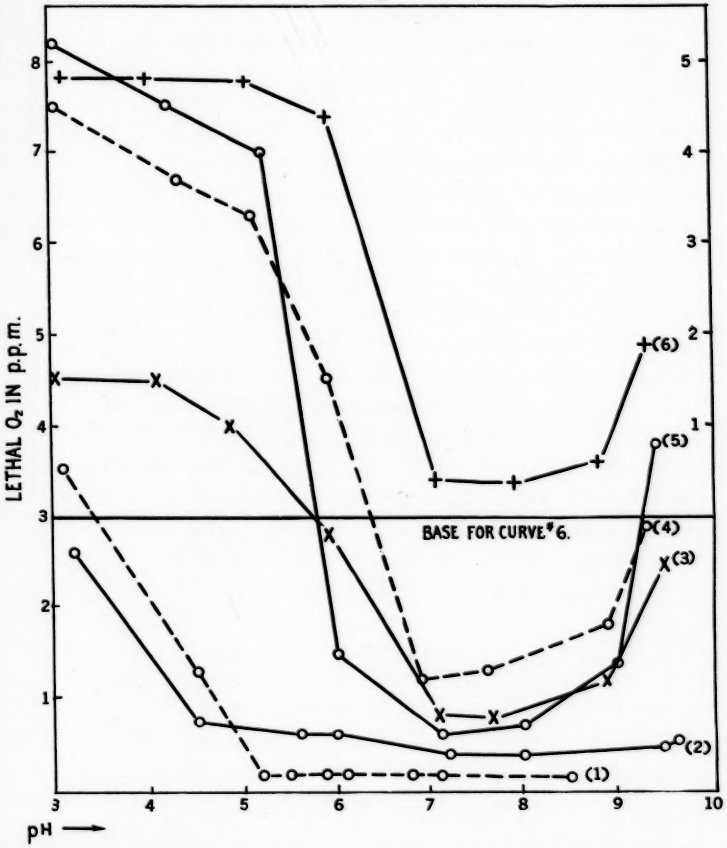

FIG. 1.—Graphs showing variations in lethal oxygen at different hydrogen-ion concentrations. (1) goldfish at 20.0° C., initial oxygen 5.1 p.p.m.; (2) yellow perch at 25° C., and initial oxygen 5.7 p.p.m.; (3) bluntnose minnow at 18° C., and initial oxygen 5.1 p.p.m.; (4) bluntnose minnow at 19° C., initial oxygen 8.2 p.p.m.; (5) steel-colored minnow at 18° C., initial oxygen 9.0 p.p.m., (6) bluegill at 20° C., initial oxygen 5.1 p.p.m.

species cannot live under low oxygen tension—likely not at any oxygen tension.

Perca flavescens Mitchill (2.5-inch fingerlings).—Like the preceding species the perch (Fig. 1) also has a wide pH range at which it

Fig. 2.

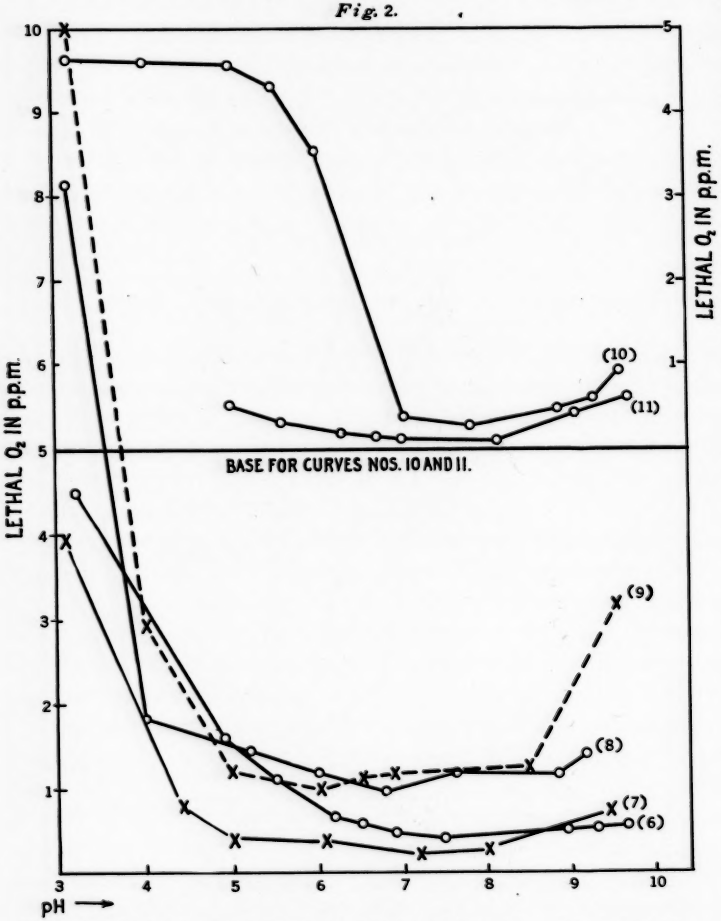

FIG. 2.—Graphs showing variations in lethal oxygen at different hydrogen-ion concentrations. (6) Smallmouth bass at 25° C., initial oxygen 7.0 p.p.m.; (7) white crappie at 25° C., and initial oxygen 5.77 p.p.m., (8) rainbow trout (5–6 inches) at 11.7° C., initial oxygen 11.0 p.p.m.; (9) rainbow trout (1.5–2 inches) 13.5° C., and initial oxygen 11.5 p.p.m.; (10) green sunfish at 20° C., and initial oxygen 5.2 p.p.m.; (11) golden shiners at 13.5° C., and initial oxygen 5.5. p.p.m.

can reduce the dissolved oxygen to a low value. The minimum seems to lie around pH 7.0–8.0, but the lethal oxygen at 9.5 is only 0.1 p.p.m. higher than at 7.2. Even at pH 5.6 the lethal oxygen is only 0.6 p.p.m.

Pomoxis annularis Rafinesque (2-inch fingerlings).—This species (Fig. 2) also exhibits a marked tolerance toward difference in hydrogen-ion concentration. The ability to extract oxygen at low pressure seems at its greatest at pH 7.1–8.0. However, if a value had been obtained at 8.5 or even at 9.0 the lethal oxygen would probably not be much greater than it is at 8.0. On the whole, this species can use the dissolved oxygen at low pressure approximately equally well over the pH range 5.5–8.0 (and probably beyond 8.0). This species as well as the perch, smallmouth black bass, and golden shiner exhibit a greater degree of tolerance on the acid side of the pH spectrum than we had anticipated. An experiment performed on another occasion showed definitely that the perch, crappie, and bass were distinctly more tolerant to acid water than the blue gill.

Erogala whipplii Girard (adults).—This species (Fig. 1) seems to be able to extract oxygen best at pH 7.0–8.0. Beyond this range the ability of this species to utilize the dissolved oxygen decreases very rapidly so that at pH 9.0 and at pH 6.0 the lethal oxygen is in excess of 1.0 p.p.m. This minnow exhibits far less tolerance to differences in pH than any of the species studied, with the exception of the bluntnosed minnow.

Hyborhynchus notatus Rafinesque (adults).—This species exhibits about the same degree of tolerance as the preceding species of minnow, only the lethal oxygen here is somewhat higher than is the case for the steel-colored minnow. However, since there is considerable variation in the minimum lethal oxygen for this species, it may be assumed that the difference in these two species of minnow are more apparent than real (Fig. 1).

Carassius auratus L. (3–4-inch fingerlings).—This species seems unusually tolerant to differences in pH (Fig. 3). This species is able to reduce the oxygen to approximately 0.15 p.p.m. over the entire pH range from 5.2 to 8.5 (and undoubtedly beyond that) (Fig. 1).

Salmo shasta Jordan.—Individuals of this species of two size groups, 1.5–2.0 inches and 5–6 inches, show a marked tolerance to

differences in hydrogen-ion concentrations. The larger fish show very little difference in the lethal oxygen from pH 5.0 to pH 9.1, and the smaller fish from pH 5.2 to pH 8.5. At pH 9.5 there is a marked increase in the lethal oxygen for the small fish (Fig. 2).

Fig. 3.

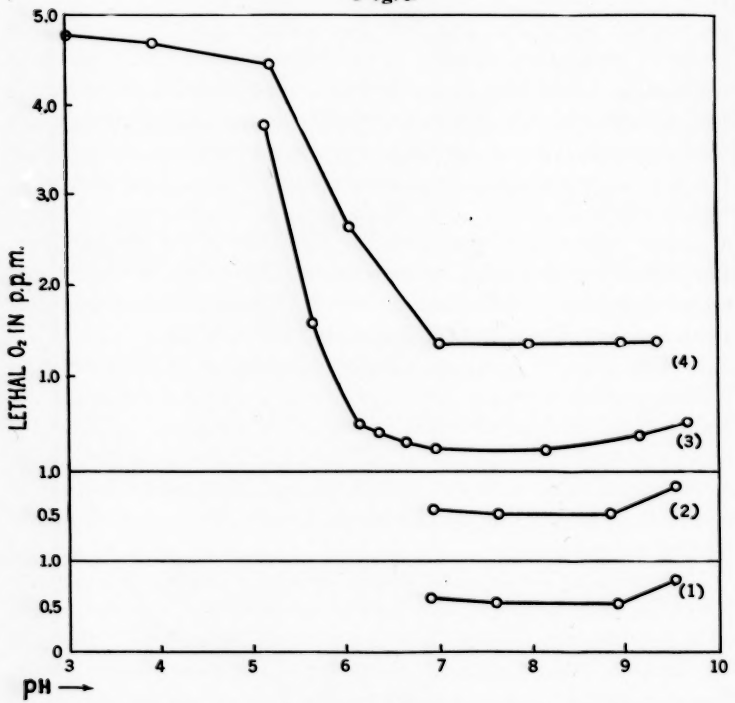

FIG. 3.—Graphs showing variations in lethal oxygen at different hydrogen-ion concentrations for largemouth bass. (1) Initial oxygen 9.0 p.p.m., temperature 19° C.; (2) initial oxygen 5.5 p.p.m., temperature 17° C.; (3) initial oxygen 5.5. p.p.m., temperature 13.5° C.; (4) initial oxygen 5.2 p.p.m.; Temperature 19° C.

Huro floridana Le Sueur (2.5–3.5-inch fingerlings).—All the curves (Fig. 3) show that this species can tolerate an oxygen equally well over the pH range 7.0 to approximately 9.0. From 9.0 to 9.5 there is a noticeable increase in the amounts of oxygen left when the fish

expires. The curves show that at 13.5° C. this species can use oxygen almost equally well at pH 6.3 and 9.1 and at 6.1 as well as at 9.6. Below pH 6.0 there is a very marked increase in the lethal oxygen. The difference for the lethal oxygen values as shown by curves at 19.0° C. and at 13.5° C. may be due to the difference in temperature. Preliminary experiments have shown that the temperature may affect the lethal oxygen. Some of the difference, we think, is due to a difference in behavior of the two lots of fish.

It may be noted here that all species of fish show violent motor activity preceding death in highly alkaline water whereas in strongly acid water they die without such activity. Also, fish that have been kept in water with a normal pH until all movements have ceased because of lack of oxygen can be revived if transferred to water having a high oxygen content. Fish that have ceased movement in either strongly alkaline or strongly acid water *cannot be revived.*

SUMMARY AND CONCLUSIONS

1. Pruthi's observations on the stickleback at pH 3.1 have been confirmed for the stickleback as well as several other species of fresh-water fish (Table II).

2. Pruthi's criticism of the work of Powers is invalid within the pH range to which the fish are acclimated. The initial oxygen has either no effect on the lethal oxygen or the effect is in the opposite direction from that suggested by Pruthi (Table I).

3. Outside of the pH range normal to the species in question the lethal oxygen is dependent in a large measure on the initial oxygen (Tables I and II).

4. Several species of fresh-water fish—largemouth blackbass, smallmouth blackbass, white crappie, yellow perch, rainbow trout, as well as the goldfish and green sunfish—have the ability to extract oxygen from the water at low oxygen tensions equally well over a fairly wide range of hydrogen-ion concentration. The bluegill has a somewhat narrower range of toleration (Figs. 1–3).

5. The two species of minnow—steel-colored and bluntnose—tolerate a markedly narrower range of difference in hydrogen-ion concentration as shown by the lethal oxygen.

6. The ability of fish to extract oxygen from the water at low pres-

sure depends more or less on the hydrogen-ion concentration of the water (Figs. 1–3).

7. The results obtained in this investigation confirm the studies on pH tolerance of fish previously published (Wiebe, 1931).

8. These results also confirm the observations (Wiebe, 1931) that in highly alkaline water fish require a higher concentration of oxygen to survive.

LITERATURE CITED

POWERS, E. B. 1921. The physiology of the respiration of fishes in relation to the hydrogen-ion concentration of the medium. Jour. Gen. Phy., **4**:305–317.

PRUTHI, HERR SINGH. 1926–27. The ability of fishes to extract oxygen at different hydrogen-ion concentrations of the medium. Jour. Marine Biol. Assoc. Unit. Kingdom. **14**:741–747.

WIEBE, A. H. 1931. Notes on the exposure of several species of pond fishes to sudden changes in pH. Trans. Amer. Mic. Soc. **50**:380–393.

OXYGEN CONSUMPTION IN THE MALE AND FEMALE FLY, DROSOPHILA MELANOGASTER[1]

(One figure)

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MANY of the investigations conducted on the fruit fly, *Drosophila melanogaster*, have been done in genetical studies, and relatively few physiological studies have been made.

Bodine and Orr (1925) made investigations on the rate of oxygen consumption at different intervals in the life of wild and vestigial *Drosophila*. They found that there was a decrease in the rate of oxygen consumption from the beginning of pupation to the end of the second day, and from that period there was an increase in the rate of oxygen consumption to the time of emergence of the imago. In studying the respiratory metabolism of the pupae, they found no physiological difference between sexes.

Clare (1925) determined the rate of oxygen consumption of the pupae of *Drosophila* and also found no significant differences in the rate of metabolism between the sexes. He states, however, that there were certain factors which suggested a difference in metabolism between the sexes. Among these factors were (1) the fact that female flies emerge earlier than the males from the pupae; (2) there was a tendency for larvae destined to become females to pupate earlier than those which would develop into males; (3) the pupae which were destined to become females had a tendency to be heavier in weight than those which were to become males.

Eddy (1931), in studying the rate of recovery from immersion in water, has shown that the females in *Drosophila* have a slightly high-

[1] Studies from the Zoölogical Laboratory, University of Nebraska, No. 178.

The author wishes to express his appreciation to Professor D. D. Whitney, under whose direction the investigations were conducted, for the many suggestions of value offered during this work.

er rate of metabolism than the males. In the Japanese beetle he found that in August the metabolic rates of the two sexes were about equal but in September the females showed a higher rate.

MATERIALS AND METHODS

A normal, red-eyed strain of flies, *Drosophila melanogaster*, the so-called wild type, was used in these experiments. The stock was obtained from the Department of Agronomy, Cornell University. The flies were kept and bred in one-half pint milk bottles, and fed the banana-agar-yeast mixture as used by many investigators. Paired matings of young male and virgin female flies were made each day and put into newly prepared culture bottles. These culture bottles containing the mated flies were kept in an electric incubator, which was adjusted and controlled at 26° C. The temperature of the incubator was the same as that of the water bath in which, later, the experiments were made. Thus, after the experiments were started, each day young flies were emerging from the pupa cases, which insured one of having flies of the same age to be used in the experiments. However, when the pupae appeared on the paper in the culture bottles, they were carefully removed and each pupa was placed separately on a small piece of moistened towelling paper, to prevent its desiccation, and put into a small vial.

This segregation of the pupae prevented the emerging flies from mating after they had emerged from the pupa cases, and also prevented them from eating, which would have affected their metabolism when the experiments were made. These vials were labeled, stoppered with a cotton plug, and placed in the electric incubator. On the next day after the flies emerged from the pupa cases their oxygen consumption was measured.

Fifty individual males and fifty individual females of the same age were used in the experiments to determine the amount of oxygen consumption, and then later the amount of oxygen consumed by each individual of both lots was computed.

After the experiments were completed, it was found necessary to obtain the average weight of control male and control female flies, since the rate of respiratory metabolism is expressed in terms per gram body-weight per unit of time. It was necessary, therefore, to

weigh two hundred and fifty male and two hundred and fifty female control flies. The flies were weighed in fifty lots of five flies of each sex, and the average weight per fly of each lot of five was determined.

Due to the small size of the flies, it was necessary to use an apparatus sufficiently sensitive to enable the recording of minute quantities of oxygen consumed when individual flies were tested. In the investigations the apparatus employed for the determination of oxygen consumption was Thunberg's microrespirometer, modified slightly, at first by Fenn and later by Obreshkove. It consists of two small bottles, each having a capacity of 12 cubic centimeters; one bottle is used as an animal chamber and the other as a compensation chamber. The two bottles are connected by a horizontal capillary index tube. The capillary index tube is graduated in the millimeter scale to a total length of 130 mm., and a bore of 0.52 mm. The capillary index tube contained a minute drop of filtered kerosene oil of 1 millimeter in length, which served as a manometer fluid and marker. The shift of the position of the drop recorded the reduction in oxygen pressure in the bottle. By the absorption of oxygen in the animal chamber, the oil index drop is caused to move toward the vessel containing the animal. Between the horizontal capillary index tube and each bottle there is a three-way stopcock, which makes it possible to connect the bottles either directly to the capillary index tube or to the outside. Each bottle is provided with a glass spoon or bulb attached to the base of the stopper. In one of these bulbs was placed the individual fly whose rate of oxygen consumption was to be measured. The bulb was small enough to assure that all possible muscular movements of the fly were at a minimum.

The experimental procedure employed was as follows: A fly was slightly etherized, placed in the bulb in the animal chamber bottle, and covered with a small piece of cotton to prevent its escape and also to aid in keeping its muscular movements at a minimum.

The small drop of kerosene oil of 1 millimeter was placed in the capillary tube and brought as close to the center of the capillary tube as possible. Then 1 cubic centimeter of a 2 per cent sodium hydroxide solution was put into each of the chamber bottles to absorb the carbon dioxide.

After the fly had been placed in the bulb in the animal chamber bottle, the stopcocks were turned so that both bottles were made to communicate with the outside. Then the apparatus was immersed

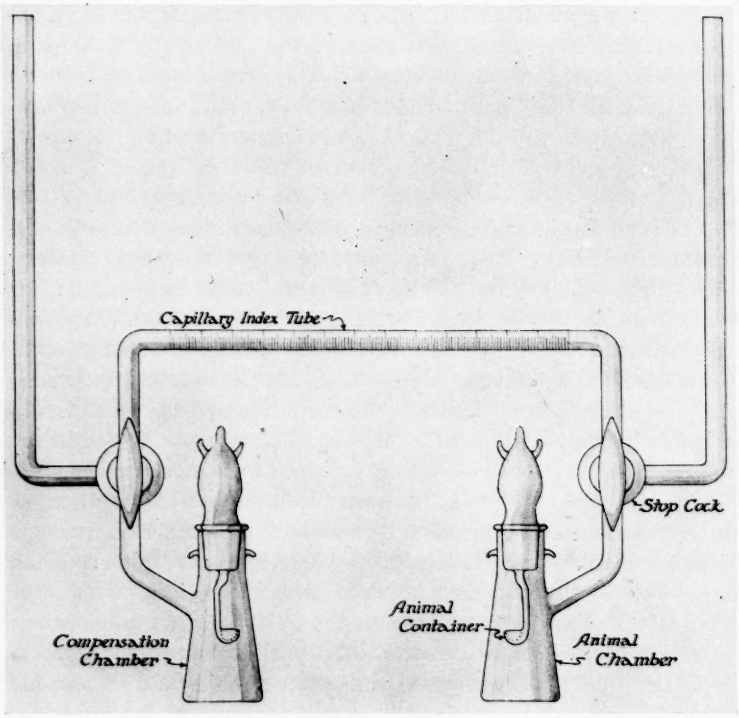

FIG. 1.—Microrespirometer of Thunberg, modified by Fenn and Obreshkove

in a Freas water bath in which the thermostat was set for 26° C, and this temperature was maintained and controlled within 0.5° C.

The microrespirometer was left in the bath for a period of ½ hour until the apparatus had come to a temperature equilibrium. Then the microrespirometer was taken out of the water bath quickly, and the stopcocks turned so that both bottles communicated only with the capillary index tube. The microrespirometer was again im-

mersed in the water bath. An additional 15-minute period of time was allowed before the position of the kerosene oil drop was recorded, thus insuring no disturbance of the kerosene oil drop from the turning of the stopcocks. After this period, when the apparatus had reached its temperature equilibrium, the position of the index drop was read at 15-minute intervals, for a period of 1 hour. Sometimes the same individual fly was subjected to observation for 2 consecutive hours, and each time it was observed there was shown a uniform rate of movement of the index drop. In other words, repeated experiments at regular intervals with the same fly in the respiratory chamber showed a uniform rate of oxygen consumption.

In calculating the absolute amounts of oxygen used by the fly, from a microrespirometer reading of 1 mm. in the capillary index tube, it was necessary to employ the following formula given by Krogh (1916):

$$Ap.\frac{273}{273+tw}+V\ .$$

RESULTS

Since the rate of metabolism is expressed per milligram of body-weight per unit of time, it was necessary to weigh five hundred control flies, similar to those used in the experiments. Two hundred and fifty flies of each sex were weighed. They were of the same age and reared under the same conditions as those which were used in the experiments. In Tables I and II are given the weights in milligrams of the control flies in lots of fives. By weighing two hundred and fifty control flies of each sex in groups of five individuals, a more accurate weight of the flies was obtained than if each fly had been weighed separately. Comparing the average weights of the groups of the control males with the average weights of the groups of control females it is seen that the average weight of each male fly was 0.88 milligram, while the average weight of each female fly was 1.1 milligrams.

In Tables I and II are given the manometer readings in millimeters for periods of 1 hour of fifty male and female flies. The average manometer reading for the males is 9.2 mm. per hour per fly,

that for the females 14 mm. per hour per fly; that is, the females show a higher average than the males.

In Column 4 of Tables I and II are given the quantities of oxygen consumed. The average quantity consumed by each of the fifty

TABLE I

MANOMETER READINGS PER HOUR FOR MALE FLIES AND OXYGEN CONSUMPTION PER MILLIGRAM BODY-WEIGHT PER HOUR PER MALE FLY CALCULATED BY USING THE AVERAGE WEIGHT OF CONTROL MALES IN LOTS OF FIVE INDIVIDUALS

Serial Number	Manometer Reading in Mm. Per Hour Per Fly	Average Weights in Mg. of 5 Male Control Flies	Oxygen Consumed in Mg. Per Hour Per Mg. Body-Weight of Fly	Serial Number	Manometer Reading in Mm. Per Hour Per Fly	Average Weights in Mg. of 5 Male Control Flies	Oxygen Consumed in Mg. Per Hour Per Mg. Body-Weight of Fly
1	8	0.8	0.002668	26	8	0.8	0.002668
2	8	0.8	0.002668	27	7	0.84	0.002223
3	8	0.8	0.002668	28	10	0.9	0.002963
4	8	0.84	0.002540	29	9	0.84	0.002858
5	10	0.9	0.002963	30	10	0.9	0.002963
6	9	0.84	0.002858	31	9	0.88	0.002728
7	10	0.9	0.002963	32	9	0.88	0.002728
8	9	0.84	0.002858	33	10	0.92	0.002900
9	9	0.86	0.002792	34	10	0.92	0.002900
10	10	0.96	0.002779	35	9	0.84	0.002858
11	10	0.92	0.002900	36	9	0.84	0.002858
12	9	0.84	0.002858	37	9	0.84	0.002858
13	9	0.84	0.002858	38	10	0.9	0.002963
14	10	0.96	0.002779	39	10	0.9	0.002963
15	8	0.8	0.002668	40	9	0.9	0.002668
16	9	0.84	0.002858	41	9	0.96	0.002501
17	10	0.92	0.002900	42	9	0.84	0.002858
18	8	0.8	0.002668	43	8	0.8	0.002668
19	10	0.92	0.002900	44	10	0.9	0.002963
20	10	0.9	0.002963	45	10	0.9	0.002963
21	9	0.88	0.002728	46	10	0.92	0.002900
22	10	0.92	0.002900	47	9	0.9	0.002668
23	10	0.92	0.002900	48	9	0.9	0.002668
24	9	0.84	0.002858	49	10	0.9	0.002963
25	9	0.96	0.002501	50	9	0.9	0.002668
Summary and average				50	9.2	0.88	0.002798

male flies was 0.002798 milligrams per milligram body-weight of fly per hour. The average quantity of oxygen consumed by each of the fifty females was 0.003386 milligrams per milligram body-weight of fly per hour. The female fly consumes on an average 0.000588 of a

milligram more of oxygen per milligram body-weight per hour than the male. In these computations the manometer reading of each fly was used together with the average weight of the flies in one of the fifty control groups. For example, the manometer reading of the

TABLE II

MANOMETER READINGS PER HOUR FOR FEMALE FLIES AND OXYGEN CONSUMPTION PER MILLIGRAM BODY-WEIGHT PER HOUR PER FEMALE FLY CALCULATED BY USING THE AVERAGE WEIGHT OF CONTROL FEMALES IN LOTS OF FIVE INDIVIDUALS

Serial Number	Manometer Reading in Mm. Per Hour Per Fly	Average Weights in Mg. of 5 Female Control Flies	Oxygen Consumed in Mg. Per Hour Per Mg. Body-Weight of Fly	Serial Number	Manometer Reading in Mm. Per Hour Per Fly	Average Weights in Mg. of 5 Female Control Flies	Oxygen Consumed in Mg. Per Hour Per Mg. Body-Weight of Fly
1.....	14	1.08	0.003458	26.....	15	1.12	0.003573
2.....	14	1.04	0.003591	27.....	13	1.12	0.003096
3.....	13	1.08	0.003208	28.....	13	1.08	0.003208
4.....	13	1.04	0.003335	29.....	14	1.12	0.003335
5.....	14	1.04	0.003591	30.....	13	1.15	0.003016
6.....	14	1.04	0.003591	31.....	15	1.10	0.003637
7.....	13	1.15	0.003016	32.....	14	1.12	0.003113
8.....	15	1.12	0.003573	33.....	15	1.10	0.003637
9.....	15	1.12	0.003573	34.....	13	1.04	0.003335
10.....	14	1.16	0.003220	35.....	15	1.12	0.003573
11.....	15	1.12	0.003573	36.....	14	1.16	0.003220
12.....	15	1.1	0.003637	37.....	15	1.16	0.003450
13.....	13	1.04	0.003335	38.....	14	1.10	0.003396
14.....	15	1.04	0.003848	39.....	13	1.04	0.003335
15.....	13	1.1	0.003153	40.....	13	1.12	0.003096
16.....	15	1.2	0.003335	41.....	14	1.16	0.003220
17.....	14	1.16	0.003220	42.....	14	1.12	0.003335
18.....	15	1.10	0.003637	43.....	15	1.10	0.003637
19.....	15	1.10	0.003637	44.....	14	1.12	0.003335
20.....	13	1.10	0.003153	45.....	13	1.15	0.003016
21.....	15	1.16	0.003450	46.....	13	1.12	0.003096
22.....	15	1.10	0.003637	47.....	15	1.12	0.003573
23.....	14	1.16	0.003220	48.....	13	1.04	0.003335
24.....	13	1.04	0.003335	49.....	14	1.08	0.003458
25.....	15	1.12	0.003573	50.....	14	1.10	0.003396
Summary and average.....				50.....	14	1.10	0.003386

male fly No. 8 was used in the computation with the average weight of a male fly in control group No. 8.

It is evident that the female *Drosophila melanogaster* has a higher rate of oxygen consumption than the male.

DISCUSSION

Bodine and Orr have made measurements of oxygen consumption upon the wild and vestigial pupae of *Drosaphila*, and they found an increase in the rate of oxygen consumption at the time of emergence of the flies. They also found that the vestigial pupae had a higher rate of metabolism than the wild type, and they suggested that this may be due to the fact that the vestigial pupae weighed more than the wild type.

Krogh (1916) working with the meal-worm, *Tenebrio*, stated that the rate of metabolism is proportional to the amount of organized tissue present and actively respiring. According to this assumption, the sex difference may be due to a greater amount of organized tissue in the female fly than in the male.

Further suggestion was given by Taylor and Steinbach (1931), who stated that at the time of emergence from the pupae, female moths were more mature sexually than the males, and this suggests the possibility that the female has relatively more organized tissue than the male at the time of emergence from the pupa, and that this may cause the difference in the rate of metabolism between the sexes.

Taylor and Steinbach (1931) also observed a sex difference in metabolism in both blow-flies and bee-moths, and they found that at the end of development all the female blow-flies had a higher rate of metabolism than the males. In the bee-moth Taylor also found a higher rate of oxygen consumption in the female than in the male. The conclusion given is that a sex difference is evident at the end of development, and at this time the females attained a higher rate of oxygen consumption than the males.

In these present experiments the determination of oxygen consumption was made on individual adult flies of the same age and not upon a number of flies collectively as has been done by previous workers. There are some objections to the latter method, in cases where the pupae are used, in that the pupae of one group, although all are of the same age, may not have the same metabolic rate at a given time, due to individual variations in development as well as other factors. The rate of oxygen consumption of some pupae might

be increasing, while the rate of others might be decreasing, and hence it is difficult to discover the changes in rate of oxygen consumption which are of short durations or which are not very pronounced. To avoid any such varying changes in the present investigations the measurements have been made upon single individual adult flies.

The results support and extend those of Eddy (1931), who also worked with adult male and female *Drosophila*, but who used a method which did not show the actual differences in terms of oxygen consumed.

SUMMARY

1. In *Drosophila melanogaster* measurements of oxygen consumption were made on fifty male and fifty female flies individually for a period of 1 hour each.

2. The rate of oxygen consumption of the female flies exceeds that of the male flies. The males consumed on an average 0.002798 milligrams of oxygen per hour per milligram body-weight of the fly, while the female flies consumed on an average 0.003386 milligrams.

3. The difference in the rate of oxygen consumption between the two sexes is 0.000588 of a milligram of oxygen per milligram body-weight per hour per fly.

4. Although the differences in oxygen consumption is small, nevertheless it indicates that there is a higher rate of metabolism in the female than in the male fly.

LITERATURE

BODINE, J. H. 1921*a*. Rate of metabolism of certain *Orthoptera*. Jour. Exp. Zoöl., **32**:137.

———. 1929. Respiratory metabolism of a developing egg. Physiol., **2**:459.

BODINE, J. H., and ORR, P. R. 1925. Physiological studies on respiratory metabolism. Biol. Bull., **48**:1.

CLARE, M. R. 1925. A study of oxygen metabolism in *Drosophila melanogaster*. *Ibid.*, **49**:440.

EDDY, M. W. 1931. Recovery from immersion in water. An index of metabolism and the condition of the gonads in *Drosophila melanogaster* and *Popillia japonica*. Jour. Morph., **51**:435.

FENN, W. O. 1928. A new method for the determination of minute amounts of carbon dioxide and oxygen. Amer. Jour. Physiol., **84**:110.

FINK, D. 1925. Metabolism during embryonic and metamorphic development of insects. Jour. Gen. Physiol., **7**:527.

KROGH, A. 1915. Über Mikrorespirometrie. Handbuch der Biochemischen Arbeits Methoden, **8**:519.

———. 1916. The respiratory exchange of animals and man. London and New York.

LOEB, J., and NORTHROP, J. H. 1917. On influence of food and temperature upon the duration of life of Drosophila melanogaster. Jour. Biol. Chem., **32**:103.

MELVIN, R. 1918. Oxygen consumption of insect eggs. Biol. Bull., **55**:135.

NORTHROP, J. H. 1926. Carbon dioxide production and duration of life of *Drosophila* cultures. Jour. Gen. Physiol., **9**:319.

OBRESHKOVE, V. 1930. Oxygen consumption in the developmental stages of a cladoceran. Physiol. Zoöl., **3**:271.

OBRESHKOVE, V., and BANTA, A. M. 1930. A study of the rate of oxygen consumption of different Cladocera clones derived from a single mother. *Ibid.*, **3**:1.

ORR, P. R. 1925. A critical thermal increment for oxygen consumption of an insect, *Drosophila melanogaster*. Jour. Gen. Physiol., **1**:731.

PEARL, R., and PARKER, S. L. 1922. Experimental studies on the duration of life. Amer. Nat., **55**:481.

SAYLE, M. H. 1928. The metabolism of insects. Quar. Rev. Biol., **3**:542.

TAYLOR, I. R. 1927. Oxygen consumption of individual pupae during metamorphosis. Jour. Morph. and Physiol., **44**:313.

TAYLOR, I. R., and STEINBACH, H. B. 1931. Respiratory metabolism during pupal development of bee-moth. Physiol. Zoöl., **4**:604.

THE EFFECT OF OXYGEN TENSION ON OXYGEN CONSUMPTION OF A DEVELOPING EGG (ORTHOPTERA)

(Two figures)

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INTEREST in the question of the effects of oxygen tension on oxygen consumption in cells and organisms has curiously centered largely around aquatic forms. With the exception of vertebrates the major part of our information on this subject seems to have come from studies on aquatic invertebrates (Krogh, 1916; Hyman, 1929). Little, if any, information seems to exist for air-breathing insects. Luciani and Piutti (1888), working on the eggs of the silk worm, report that for hibernating eggs the oxygen consumption is directly proportional to the partial pressure of oxygen. (These authors apparently used but three concentrations of gas, pure oxygen, air, and nitrogen.) Thunberg (1905) for the meal worm, Gaarder (1918) for the pupa of *Tenebrio*, v. Buddenbrock and Rohr (1922) for the walking-stick, and Cook (1932) for the termite, all report that oxygen intake is more or less independent of oxygen tension within rather wide limits. The present paper deals with results of investigations on the effects of oxygen tension upon oxygen consumption of the developing egg of the common grasshopper, *Melanoplus differentialis*.

As pointed out in a previous publication (Bodine, 1932), the grasshopper (*M. differentialis*) lays two types of eggs, one exhibiting a diapause or developmental block, the other lacking it. Both types of eggs have been separately used in this investigation. Comparable morphological and developmental stages, from the so-called "three week stage" (Slifer, 1932) to hatching, have been employed for both varieties. Oxygen determinations were carried out by means of the Krogh manometer (Bodine, 1929) at constant temperature of 25° C. Various numbers of eggs, ranging from five to one hundred and twenty-five, were used in individual experiments. In any experi-

ment the same manometer was used for both normal (air) and experimental determinations. Different tensions of oxygen were made with tank nitrogen (99.7 per cent) and oxygen over water in a large calibrated glass cylinder. Gas mixtures were slowly passed through the side arms of manometer vessels, thus insuring complete filling of the vessel with the mixture in question. All oxygen consumption

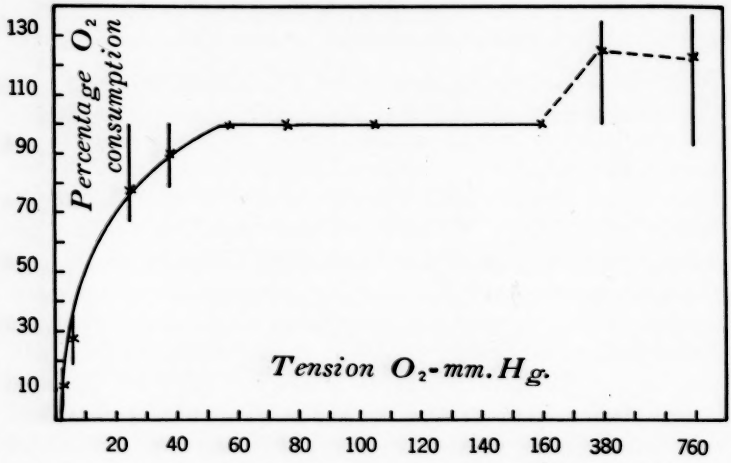

FIG. 1.—Shows rates of oxygen consumption for eggs at different tensions of oxygen. Solid line from 160 mm. and below, for diapause and non-diapause eggs. Dotted lines at 380 mm. and 760 mm. for non-diapause eggs. Abscissae, tensions oxygens in mm. mercury. Ordinates, percentage oxygen consumption with air (160 mm.) taken as 100 per cent. Crosses indicate average values for series of readings. Vertical lines through crosses indicate range for each series. For further description see text.

determinations, both in air and experimental mixtures, extended over 2–3-hour periods. For each tension of gas tested, at least ten to twenty-five separate determinations were carried out. In most cases, but one tension of gas, in addition to air, was used for each set of eggs, since it was early found that recovery from lower tensions was, in many cases, quite slow. Results are expressed in relation to rate of oxygen consumption in air as one hundred per cent.

Results for both diapause and non-diapause eggs are shown graphically in Figures 1 and 2. From an inspection of Figure 1 it will be

noted that for tensions of oxygen of approximately 25 mm. to 160 mm. of mercury the oxygen consumption for both diapause and non-diapause eggs is quite independent of the external oxygen tension. It is only when the tensions of oxygen fall below 25 mm. that the rates of oxygen consumption are appreciably affected.

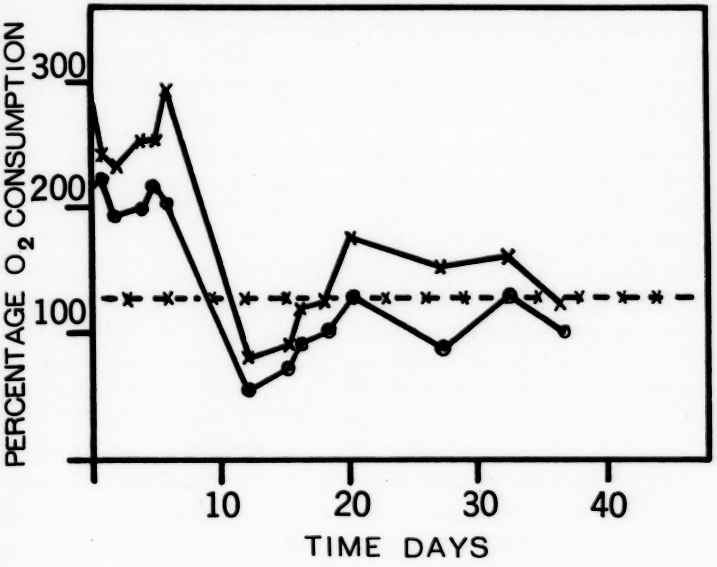

FIG. 2.—Shows rates of oxygen consumption for a typical series of diapause eggs at oxygen tensions of 380 mm. and 760 mm. mercury. Solid line for diapause eggs. Dotted line average for non-diapause eggs as control. Abscissae, time in days indicated from beginning of diapause. Ordinate, percentage oxygen consumption with air (160 mm.) taken as 100 per cent. Crosses (×) indicate rates of oxygen consumption in oxygen tension of 380 mm. Circles (O) indicate rates for 760 mm. tension. For further description see text.

In tensions of 380 mm. and 760 mm. stimulating effect on the oxygen consumption is invariably noted (Figs. 1 and 2). During the 2–3-hour experimental periods no injurious effects of the exposures to high tensions of the gas were detected. Significant differences, however, in responses to high oxygen tensions by diapause and non-diapause eggs were found. Non-diapause eggs, or those in which

the diapause block had been destroyed and development was proceeding in a regular and normal manner, are stimulated when exposed to atmospheres of 50 per cent to 100 per cent oxygen, as shown in Figure 1. The degree of stimulation during a 2-hour exposure is quite definite and reaches an average value of 125–130 per cent. If the length of exposure is prolonged a gradual injurious effect is noted, and the rates of oxygen consumption are depressed. Diapause eggs, or those in a state of developmental block, respond to high oxygen tensions in a varying manner, apparently dependent on the physiological state at the time of application of the gas. Physiologically, diapause eggs are rather unstable. Their rates of oxygen consumption are decidedly lower than for non-diapause eggs in comparable morphological stages (Bodine, 1932). In the early stages of diapause, reactions to high oxygen tensions are pronounced, at times reaching values as high as 300 per cent (Fig. 2). As diapause proceeds the reaction becomes less pronounced, until in late diapause it resembles that for normal growing eggs. The rather variable physiological nature of diapause makes its evaluation in quantitative terms quite impossible. As a general rule, however, eggs in early stages of diapause react greatest, while during successively later periods the reaction approaches that for normal growing eggs.

Judging from the present results for the egg of the grasshopper, together with those previously cited for other insects, it would seem that these organisms fall into that group wherein oxygen consumption is normally quite independent of oxygen tension within rather wide ranges.

CONCLUSION AND SUMMARY

1. A study has been made of the effects of oxygen tension on oxygen consumption of the egg of an insect, the grasshopper, *Melanoplus differentialis*. 2. Within rather wide ranges, the oxygen consumption is independent of the oxygen tension of the environment. 3. No significant differences in reaction between non-diapause and diapause types of eggs have been noted for tensions below 160 mm. mercury. 4. Eggs in diapause show marked reactions to high tensions of oxygen (380 mm. and 760 mm. mercury), the degree of reaction being apparently conditioned by the physiological condition of the eggs at the period of exposure.

LITERATURE CITED

BODINE, J. H. 1929. Factors influencing the respiratory metabolism of a developing egg (Orthoptera). Phys. Zoöl., **2**:459.

———. 1932. Hibernation and diapause in certain Orthoptera. II. Response to temperature during hibernation and diapause. Phys. Zoöl., **5**:538.

v. BUDDENBROCK, W., and G. ROHR. 1922. Die Atmung von Dixippus Morosus. Zeit. f. allg. Physiol., **20**:111.

COOK, S. F. 1932. The respiratory gas exchanges in termopsis nevodensis. Bio. Bull., **63**:246.

GAARDER, R. 1918. Über den Einfluss des Sauerstoffdruckes auf den Stoffwechsel. I. Nach Versuchen an Mehlwurmpuppen. Biochem. Zeitschrift, **89**:48–93.

HYMAN, L. H. 1929. The effect of oxygen tension on oxygen consumption in *Planaria* and some echinoderms. Phys. Zoöl., **2**:505.

KROGH, A. 1916. Respiratory exchange of animals and man. Longmans, Green & Co.

LUCIANI, L., and A. PIUTTI. 1888. Sur les phénomènes respiratoires des œufs du Bombyx du Murier (1). Archives Italiennes de Biologie, **9–10**:319.

SLIFER, E. 1932. Insect development. IV. External morphology of grasshopper embryos of known age and with a known temperature history. Jour. Morph. and Physiol., **53**:1.

THUNBERG, T. 1905. Der Gasaustausch einiger niederer Tiere in seiner Abhängigkeit vom Sauerstoffpartialdruck. Skand. Arch. Physiol., **17**:133.

IRON CONTENT AND OXIDATION RATES OF A DEVELOPING EGG (ORTHOPTERA)

(Two figures)

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WITHIN the last several years considerable interest has centered around the element iron and especially as to the possible rôle it plays in questions of cellular oxidations. Through the researches of Warburg and his school (1928, 1931) an elaborate explanation of the mechanism of cellular oxidation has been developed, and in this theory it is the element iron which is of paramount significance. The experimental materials upon which this concept has been tested out are extremely varied, ranging from simple plant cells, like yeast, to complicated mammalian tissue, such as liver. The present paper is based upon a study of the iron content and oxidation rates in developing and blocked (diapause) eggs of the common grasshopper, *Melanoplus differentialis*.

In a previous communication (Bodine, 1933) it has been pointed out that eggs of the grasshopper (*M. diff.*) are of two types—one which goes through embryological development at a uniform rate at a constant temperature, while the other develops at a similar constant rate to a definite embryological stage (3 weeks at 25° C.) (Slifer, 1932), and then goes into a condition of developmental block or diapause which is for its occurrence quite independent of environmental temperature. Rates of oxidation during development and developmental blocks (diapause), as previously shown (Bodine, 1929), stand in marked contrast with each other. During development oxygen consumption is relatively high, while during diapause, or developmental block, cellular oxidations are at a relatively constant low basal rate. The rather unique inherent oxidation control in this material affords favorable opportunity for testing out various hypotheses as to the probable rôle of an iron containing respiratory catalyst during such diverse phases of cellular activity.

Individual eggs of this species are of sufficient size that they can

be easily handled experimentally while the contained embryos from the 3-week stage and on at 25° C. are of such dimensions that they can be easily freed from the surrounding yolk. Rates of oxygen con-

TABLE I

A SUMMARY OF THE IRON DETERMINATIONS FOR WHOLE EGGS AT DIFFERENT PERIODS OF DEVELOPMENT

Number of Determinations	Number of Eggs	Stage of Development	Av. Fe Content Mgs. Per Egg	Av. Per Cent of Fe
12	120	pre-diapause (7–12 days at 25° C.)	0.0050	0.125
46	460	diapause (3 weeks at 25° C.)	0.0055	0.118
47	470	post-diapause	0.0062	0.110

Average Percentage = 0.117

TABLE II

A SUMMARY OF THE IRON DETERMINATIONS FOR WHOLE EGGS, EMBRYOS FREED FROM YOLK, AND FOR YOLK MINUS EMBRYO DURING DIAPAUSE AND POST-DIAPAUSE DEVELOPMENT AT 25° C.

Stage of Development	Fe in Mgs.		
	Whole Egg	Embryo	Yolk
diapause	0.00563	0.00140	0.00448
post-diapause:			
(3 days at 25° C.)	0.00567	0.00161	0.00401
(4 days at 25° C.)	0.00582	0.00186	0.00399
(6 days at 25° C.)	0.00549	0.00261	0.00290
(9 days at 25° C.)	0.00616	0.00294	0.00328
(11 days at 25° C.)	0.00629	0.00355	0.00268
(15 days at 25° C.)	0.00598	0.00404	0.00193
(16 days at 25° C.)	0.00594	0.00442	0.00155
ready to hatch	0.00467	0.00467	.....
ready to hatch	0.00484	0.00484	.....
ready to hatch	0.00479	0.00479	.....

sumption for different types of eggs have been carried out by means of a modified Krogh manometer (Bodine, 1929). Iron determinations have been made largely by the delicate colorimetric method of

Elvehjem and Hart (1926). The only exception to the original method as described by these authors has been that the phosphates were not removed from the eggs prior to color determinations, since they occur in such low concentrations as not to interfere with such procedures. In carrying out iron determinations whole eggs, dissected out

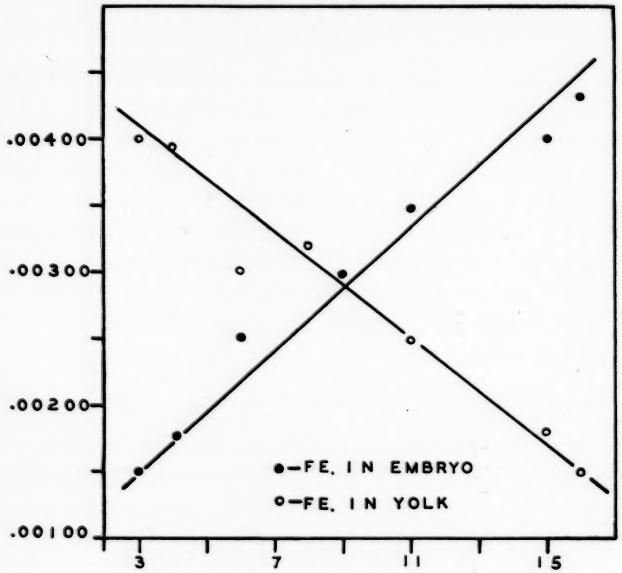

FIG. 1.—Shows relation between iron concentration of embryo and yolk during post-diapause development at 25° C. Abscissae, time in days. Ordinates, iron in milligrams. For further description see text.

embryos, and fully formed embryos have been used in groups of ten. Prior to the 3-week stage at 25° C. the embryos are so small that it is almost impossible to conveniently free them from the yolk, so that no valid determinations have been possible on embryos at these stages. All experimental eggs were taken from carefully timed lots kept at 25° C. from the time of laying. Some two thousand eggs have been used in lots of ten for each determination. Results of experiments are summarized in Tables I and II.

No significant differences in the iron content of the whole egg have

been noted during its entire course of development (Table I). This is perhaps what should be expected, since the egg is a closed system and takes in little if anything other than water (Bodine, 1933). In Table II are given values for relative amounts of iron in the whole egg, the embryo, and the yolk for different periods during embryonic development. As development proceeds the amount of iron in the embryo steadily increases, while that of the yolk decreases proportionally. In other words, as the protoplasmic mass of the embryo in-

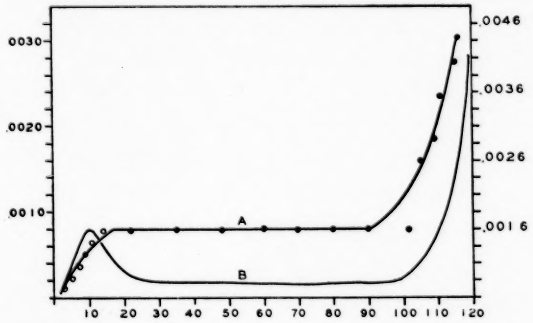

FIG. 2.—Shows relation between iron concentration of embryo and rate of oxygen consumption of egg during entire course of development at 25° C. Abscissae, time in days. Ordinates at left, rates of oxygen consumption, cc./egg/hr. Ordinates at right, iron in milligrams per embryo. Curve *A*, iron. ●=actual determinations. *O*=theoretical values. Curve *B*, rate of oxygen consumption.

creases, its iron content increases proportionally. This relationship is more clearly shown graphically in Figure 1.

The occurrence of an extended embryonic diapause or block in the development of the *M. differentialis* egg affords an excellent opportunity for the study of the possible rôle of iron in cell oxidative processes. In Figure 2 are graphically shown the relations between the iron concentrations of embryos and rates of oxidative activity during the various phases of embryonic development. During diapause, it will be noted, metabolic activity is extremely low as compared with the non-diapause or developing conditions. Iron concentrations of the embryo, on the other hand, remain extremely constant during the entire diapause period and increase only when the block to de-

velopment is removed and normal growth or activity is again resumed. During growth, or the post-diapause period, the iron content of the embryo increases in a regular fashion, closely paralleling the changes in size and metabolic rates of the embryo. If iron, according to Warburg (1928, 1931), functions as a catalyst in cell oxidations, then one might expect to note marked differences in iron concentrations or degrees of saturation in comparable morphological stages that exhibit widely varying degrees of respiration. Since it has been found that the iron concentration remains relatively constant for diapause and non-diapause embryos of the same morphological stages, it is perhaps reasonable to assume that differences in degrees of saturation of the iron exist in the two cases. In subsequent communications, on the effect of cyanides and carbon monoxide on the oxidation rates, evidence will be presented supporting the latter view.

SUMMARY AND CONCLUSIONS

1. The iron concentrations of the whole egg, embryo, and yolk of the grasshopper, *Melanoplus differentialis*, have been determined.

2. The iron concentration of the whole egg is the same (0.117 per cent) for all stages in development.

3. As the embryo develops (increases in mass) its iron content increases proportionally. Similarly, the iron content of the yolk decreases.

4. The rate of respiration of diapause and post-diapause eggs is not a function of their iron concentration, since similar iron concentrations for comparable morphological stages of both types of eggs give widely different respiratory rates.

5. Possible differences in degree of saturation of the iron acting as a respiratory catalyst in the Warburg sense have been suggested for the differences noted between diapause and non-diapause eggs.

LITERATURE CITED

BODINE, J. H. 1929. Factors influencing the respiratory metabolism of a developing egg (*Orthoptera*). Physiol. Zoöl., **2**:459.

———. 1933. The effect of hypertonic solutions on the oxygen consumption of a developing egg (*Orthoptera*). *Ibid.*, **6**:150.

ELVEHJEM, C. A., and HART, E. B. 1926. Iron in nutrition. II. Quantitative methods for the determination of iron in biological materials. Jour. Bio. Chem., **67**:43.

SLIFER, E. 1932. Insect development. IV. External morphology of grasshopper embryos of known age and with a known temperature history. Jour. Morph. and Physiol., **53**:1.

WARBURG, O. 1928. Über den Stoffwecksel der Tumoren. Berlin: J. Springer.

———. 1931. Über Nichthemmung der Zellatmung durch Blausäure. Biochem. Zeitschr., **231**:492.

(All the above contain rather complete bibliographies.)

AMOEBOID MOVEMENT IN PELOMYXA PALUSTRIS (GREEFF)

(Two figures)

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PELOMYXA PALUSTRIS is a multinucleate amoeboid animal, usually ellipsoidal in form and frequently 2–3 mm. long. Greeff (1874) was the first to describe it; he called it *Pelobius* in 1870 and *Pelomyxa palustris* in 1874. His description is remarkably full and accurate. He maintains that it consists of a thin, outer, tough, hyaline layer and a central granular structure which contains, among other things, numerous vacuoles, that the central portion moves freely back and forth, and that this movement, resulting at times in the formation of pseudopods and in locomotion, is due to localized contraction in the hyaline layer.

Schulze (1875) made a more thorough study of locomotion in this form. He agrees with Greeff in the contention that it consists of a thin outer layer "Rindenschicht" and an inner granular substance, and that movement is due to the action of the outer layer; but he maintains that a portion of the granular substance is in the form of a girdle "Gürtel" around the central part of the organism, that the rest of it flows through this girdle, proceeds until it comes in contact with the "Rindenschicht," where it spreads out in all directions, then proceeds backward at the surface until it comes in contact with the anterior edge of the girdle where it comes to rest, and that the substance in the posterior edge of the girdle becomes active, moves toward the center, and then forward through the girdle, each granule thus making a complete circuit.

Bütschli (1892) observed that particles attached to the surface of *Pelomyxa* often move in a direction precisely opposite that of those in the organism immediately beneath the surface. He concluded that this organism contains a thin surface membrane, "Häutchen." He agrees with Schulze in reference to the movement of the granular

plasma but he maintains that this plasma continuously changes to hyaline plasma at the anterior end and *vice versa* at the posterior end, and that movement is due to reduction of surface tension of the granular portion at the anterior end under the "Häutchen" and consequent forward flow in the center and backward flow at the surface of this portion.

A number of other investigators (Bourne, 1891; Penard, 1893; Gould, 1894; Wilson, 1900; Okada, 1930; *et al.*) have considered the problem of locomotion in this form but they have added nothing of importance to the views set forth above.

The specimens used in the following observations were collected at various times during July, August, and September in shallow water containing much decaying organic substance, in a marshy portion of a fresh-water pond in Falmouth, Massachusetts. These specimens were in full accord with Greeff's description of *Pelomyxa palustris*. They varied greatly in size, some being barely visible under magnification of 200 diameters and others between 2 and 3 mm. in length.

They were studied under low- and high-power lens systems, including apochromatic oil immersion objectives with magnifications up to 1,800 diameters. The observations under low magnification were made on specimens under normal conditions; those under high magnification on specimens which were flattened under a cover-glass supported with a vaseline ring. It was found that by gently pressing on the cover glass supported in this way, they can without injury be forced to spread out until they are so thin that details in structure and movement in all parts of the body can be clearly seen under the highest magnification used, and that if the spreading is not carried too far, locomotion continues.

The results of numerous observations on the process of locomotion made in this way on numerous specimens ranging in size from the smallest to the largest found are summarized in the following pages.

Pelomyxa palustris consists of a very thin outer membrane (plasmalemma), a hyaline layer under this, in places so thin that it is invisible, and a central granular mass which is very obviously alveolar in structure throughout. The outer portion of this mass (plasmagel) is more solid than the inner portion (plasmasol.) *Pelomyxa palustris* is

consequently essentially like *Amoeba proteus* in these respects. However, in *Pelomyxa* the hyaline layer at the tip of advancing pseudo-

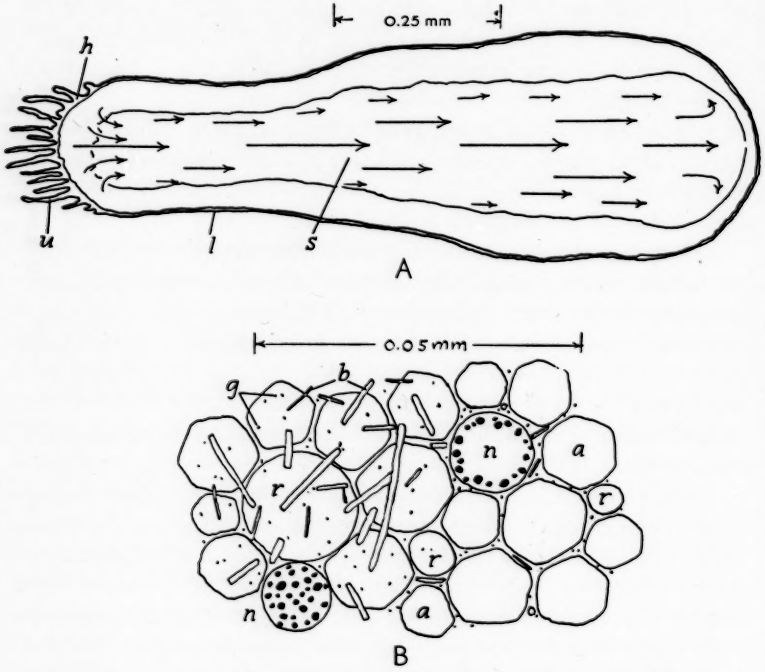

FIG. 1.—*A*, camera outline of an optical section of a small specimen of *Pelomyxa palustris* seen from above. *l*, plasmalemma; *g*, plasmagel; *s*, plasmasol; *u*, uroid; *h*, hyaline layer; arrows, direction of movement; *mm*, projected scale.

Note that the hyaline layer is very thin except in the region of the uroids, that there is no hyaline cap, and that the plasmagel is transformed into plasmasol at the posterior end and the plasmasol into plasmagel at the anterior end.

B, camera outline showing details in structure of the plasmagel. Left side, surface view; right side, optical section near surface. *a*, alveoli, *n*, nuclei, *r*, refractive bodies; *b*, rods (probably bacteria); *g*, granules.

The plasmasol is the same in structure as the plasmagel but the alveoli in it are much more nearly spherical and not so closely packed. The rods and granules are much more numerous on the outer surface of the plasmagel than elsewhere. The refractive bodies consist largely of glycogen. They vary greatly in size.

pods is very thin (Fig. 1), whereas in *Amoeba* it is thick, forming a structure known as the hyaline cap, and in the former the granular

portion, as previously stated, is distinctly alveolar, whereas in the latter it is not. When *Pelomyxa palustris* is moving freely, it is almost invariably ellipsoidal in form, having but one pseudopod with the extending tip continuously at the anterior end of the animal. In this condition the plasmagel at the tip of the pseudopod, i.e., the plasmagel sheet, is so thin that it consists of a single layer of alveoli and this is in close contact with the plasmalemma. The rest of the plasmagel is much thicker. The alveoli in this and the plasmagel sheet are closely packed together, having angular surfaces which fit into each other, and they are fairly definitely fixed in position in reference to each other, i.e., there is but little movement among them. The plasmasol is likewise composed almost entirely of alveoli, but the alveoli in it are much less angular and the spaces between them are larger than those in the plasmagel, and they roll over each other freely, i.e., they are not fixed in position in reference to each other as are those in the plasmagel. The plasmagel consequently forms a relatively firm, elongated, closed sac around the plasmasol, not merely a girdle of resting substance, as Schulze maintained.

At the inner surface of one end of this sac, the posterior end, the alveoli in the plasmagel continuously round up, then they move toward the center of the animal and forward until they reach the plasmagel sheet at the anterior end.[1] Here some of them are squeezed one at a time between the alveoli in this sheet, and as this occurs they become angular in form and fixed in position (Fig. 2). Others which come in contact with this sheet farther back adhere to it, become angular in form, and fixed in position, i.e., they become attached to each other. Thus, during locomotion the plasmagel sheet is at the tip continuously stretched and built up by the introduction of alveoli from the plasmasol and further back continuously thickened by addition to the inner surface of alveoli from the plasmasol. In other words, at the posterior end the plasmagel becomes more fluid, solates, and is thus transformed into plasmagel. There is, however, no continuous transformation of granular substance (endosarc) into hyaline substance (ectosarc) and vice versa as Bütschli maintains.

If locomotion ceases, even for only a few seconds, and then begins again, there is formed at the tip of the pseudopod between the plas-

[1] They do not reach the "Rindenschicht" as Schulze maintains.

malemma and the plasmagel sheet a very definite hyaline layer (hyaline cap), then the plasmagel sheet breaks, the plasmasol flows out into the cap, and the alveoli in it spread out, tumbling over each

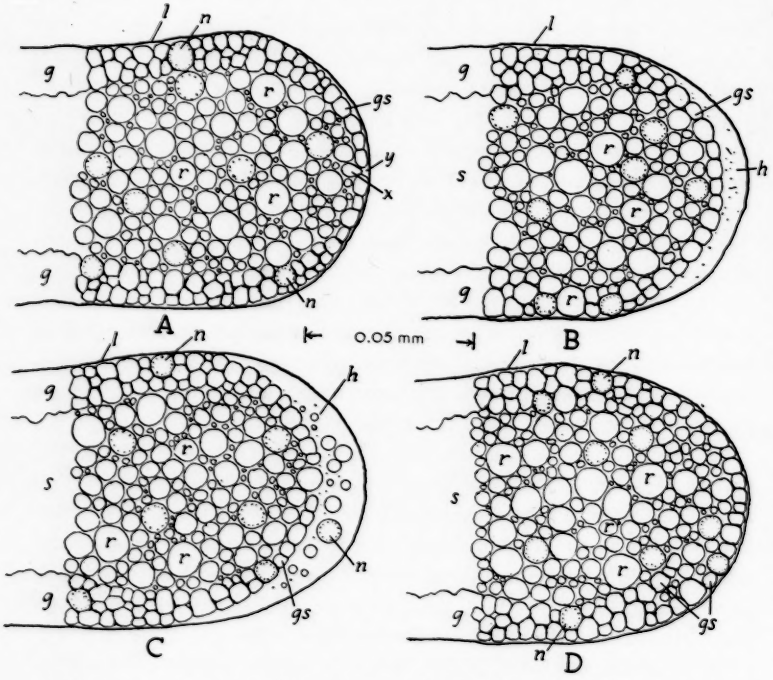

FIG. 2.—Camera outlines of the anterior end of a moving pelomyxa illustrating various phenomena involved in the process of locomotion. *l*, plasmalemma; *g*, plasmagel; *s*, plasmasol; *gs*, plasmagel sheet; *h*, hyaline cap; *a*, alveoli; *n*, nuclei; *r*, refractive bodies.

A, transformation of plasmasol into plasmagel. Note that the plasmagel sheet consists of a single layer of alveoli, that this is apparently stretched until some of the alveoli begin to separate (*y*) and that alveoli (*x*) in the plasmasol are then pushed in to fill the gaps.

B, formation of a hyaline cap by accumulation under the plasmalemma of fluid and small particles which have been squeezed through the plasmagel sheet. This always occurs before locomotion begins, even if it has stopped only a few seconds.

C, breaking of the plasmagel and flowing of the plasmasol into the hyaline cap. Note that the alveoli readily spread out in the cap, showing that its content is fluid.

D, formation of a new plasmagel sheet and disintegration of the old. After this is complete locomotion continues without a hyaline cap until it stops.

other as they proceed, until the cap is well filled with them. Those in contact with the plasmalemma soon form a definite layer, becoming angular in form and fixed in position, i.e., the plasmasol gelates and forms a new plasmagel sheet,[2] then the old plasmagel sheet disintegrates and locomotion proceeds as described above (Fig. 2).

The hyaline cap is apparently formed by the accumulation of fluid, from the plasmasol, which is squeezed through pores between the alveoli in the plasmagel sheet. The fact that, after this sheet breaks, the alveoli spread freely throughout the cap shows that the viscosity of the hyaline substance in it is relatively very low. This is not in accord with the conclusion reached by Greeff and Schulze. They contend that the hyaline layer is the toughest structure in the organism and that, as stated above, movement is due to localized contraction in it.

The factors involved in locomotion in *Pelomyxa palustris* are essentially the same as those involved in *Amoeba proteus* (Mast, 1923, 1926, 1931). *Pelomyxa* is a turgid system. The plasmagel and the plasmalemma are consequently stretched. The elastic strength of the plasmagel is lowest in the thin sheet at the anterior end, higher at the posterior end, and highest in the region between. It consequently contracts at the sides and the posterior end and simultaneously expands at the anterior end. This results in an elongated form and in forward streaming of the plasmasol. The plasmalemma is probably but little, if at all, involved in this process, but it nevertheless has an important function in locomotion. It is attached to the substratum and to the plasmagel near the posterior end; and this, owing to the stretching and the building up of the plasmagel sac at the anterior end and its contraction and breaking down at the posterior end with the forward flow of the plasmasol, results in forward movement of the entire animal, i.e., in locomotion.

The plasmalemma moves over the plasmagel as described by Bütschli but it does not move continuously during locomotion and it does not always move forward on the upper surface as he maintains. Moreover, it does not move in all regions of the surface; there is no rolling like that observed by Jennings (1904) in *Amoeba verrucosa*. It probably never slides over the plasmagel at the posterior end

[2] Contact with the plasmalemma evidently induces gelation of the plasmasol.

where there usually is a complicated series of folds, projections, and indentations, forming what is known as a uroid. The irregularity in the movement of the plasmalemma in relation to the plasmagel is doubtless due to alternate stretching and contracting of this layer in various directions, owing to its attachment to the substratum and the plasmagel at the posterior end.

Bütschli (1892) maintains that at the anterior end alveoli continuously break and release substance which lowers the surface tension and that this results in backward flow at the surface of the plasmagel and forward flow of the plasmasol.

The alveoli can, under favorable conditions, be very clearly seen during locomotion. I have repeatedly closely observed them at the anterior end in the plasmagel sheet but I have found no evidence in support of Bütschli's view that they break. Moreover, the forward flow as demonstrated above is due to stretching of the plasmagel sheet, owing to its relatively low elastic strength, and this is dependent upon the adhesion between adjoining alveoli. If this is true, surface tension plays a very insignificant rôle in the process of locomotion in *Pelomyxa palustris*.

The essential factors involved in locomotion in *Pelomyxa palustris* as in *Amoeba proteus* are (1) maintenance of turgidity of the system; (2) localized attachment of the plasmalemma to the substratum and the plasmagel; (3) transformation of plasmasol into plasmagel at the anterior end and *vice versa* at the posterior end; and (4) continuous difference in the elastic strength of the plasmagel in different regions. The control of the interaction between these factors in such a way as to form an automatically, co-ordinated, operating system constitutes the central problem in amoeboid movement.

There are, however, a number of differences between *Pelomyxa palustris* and *Amoeba proteus* that should be emphasized: (1) In *Amoeba proteus* I was unable to demonstrate an alveolar structure of the plasmagel and the plasmasol, but I observed a number of phenomena which indicate such a structure. In *Pelomyxa palustris* it is very evident that the plasmagel and the plasmasol are alveolar in structure. (2) In the former I was unable to see an intact plasmagel sheet but, owing to various observed phenomena, I postulated one. In the latter this sheet can be clearly seen. (3) In *Amoeba* I postu-

lated that this sheet is, during extension of the pseudopods, continuously stretched and that as it is stretched and becomes thinner, it is continuously built up by addition of substance from the plasmasol. In *Pelomyxa* it is very obvious that this obtains, and it can be clearly seen precisely how the sheet is built up and how uniform thickness in it is maintained. *Pelomyxa palustris* is, therefore, a very favorable form for the study of amoeboid movement.

SUMMARY

1. *Pelomyxa palustris* is ellipsoidal in form. It contains a thin, elastic, tough outer membrane (plasmalemma), a large central granular mass, and a very thin, hyaline, fluid layer between these two structures. The surface of the granular mass is relatively solid and elastic (plasmagel); the rest is fluid (plasmasol). It is distinctly alveolar in structure throughout.

2. During locomotion the plasmagel is very thin at the anterior end, consisting of a single layer of alveoli; the plasmalemma is attached to the substratum and the plasmagel near the posterior end; elsewhere it slides over the plasmagel, sometimes forward, sometimes backward; the plasmagel solates (becomes plasmasol) at the posterior end, flows to the anterior end, and there gelates (becomes plasmagel).

3. The elastic strength of the plasmagel is lowest at the anterior end, higher at the posterior end, and highest at the sides. It consequently contracts at the sides and the posterior end and pushes the plasmasol forward against the plasmagel at the anterior end (plasmagel sheet) and stretches it. This stretching separates the alveoli in it and tends to break it down, but as soon as the alveoli in this sheet separate, some of those in the plasmasol at the tip are squeezed in and a little farther back others become attached to the inner surface. Thus, the plasmagel sheet is built up as rapidly as it is broken down.

4. The principal factors involved in locomotion are: (1) localized adhesion of the plasmalemma to the substratum and to the plasmagel; (2) maintenance of turgidity in the system; (3) solation of the plasmagel at the posterior end and gelation of the plasmasol at

the anterior end; and (4) maintenance of relatively low elastic strength of the plasmagel at the anterior end.

5. The process of locomotion in *Pelomyxa palustris* is essentially the same as it is in *Amoeba proteus*, but some of the details in the process are much more obvious in the former than in the latter.

LITERATURE CITED

BOURNE, ALFRED GIBBS. 1891. On *Pelomyxa viridis* sp.n. and on the vesicular nature of protoplasm. Quart. Jour. Micr. Sci., **32**:357–74.

BÜTSCHLI, O. 1892. Untersuchungen über mikroskopische Schäume und das Protoplasma. Leipzig. Pp. 234.

GOULD, LILIAN J. 1894. Notes on the minute structure of *Pelomyxa palustris* (Greeff). Quart. Jour. Micr. Sci., **36**:295–96.

GREEFF, R. 1874. *Pelomyxa palustris* (Pelobius), ein amöbenartiger Organismus des süssen Wassers. Archiv f. Mikr. Anat., **10**:51–73.

JENNINGS, H. S. 1904. Contribution to the study of the behavior of lower organisms. Pub. Carnegie Inst. Wash. Pp. 257.

MAST, S. O. 1923. Mechanics of locomotion in Amoeba. Proc. Nat. Acad. Sci., **9**:258–61.

———. 1926. Structure, movement, locomotion and stimulation in Amoeba. Jour. Morph. and Physiol., **41**:347–425.

———. 1931. Locomotion in *Amoeba proteus* (Leidy). Protoplasma, **14**:321–30.

OKADA, YO K. 1930. Über den Bau und die Bewegungsweise von *Pelomyxa*. Arch. f. Prot., **70**:131–54.

PENARD, E. 1893. *Pelomyxa palustris*. Arch. d. Sci. Phy. et Nat., Ser. III, Vol. XXIX, pp. 166–84.

SCHULZE, F. E. 1875. Rhizopodenstudien. IV. Arch. f. Mikr. Anat., **11**:329–53.

WILSON, H. V. 1900. Notes on a species of Pelomyxa. Am. Nat., **34**:535–50.

STUDIES ON EXPERIMENTAL LACTATION IN THE RABBIT

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INVESTIGATIONS carried out within recent years have shown definitely that when extracts of the anterior pituitary glands of either beef or sheep are injected into intact or ovariectomized female rabbits and guinea pigs the mammary glands undergo considerable development and secrete milk (Stricker and Grueter, 1929; Corner, 1930; Asdell, 1931; Turner and Gardner, 1931; and Nelson and Pfiffner, 1931).

The present study was undertaken with the purpose of determining the suitability of the rabbit for the purpose of biologically assaying the lactation factor and to gain further information concerning the mechanism involved in the control of normal lactation.

With a single exception all of the anterior pituitary extracts used in the following experiments were prepared in this laboratory with the kind co-operation of Dr. J. J. Pfiffner, to whom the writer is greatly indebted. Several samples of anterior pituitary extract, theelin, and Antuitrin S, were supplied through the courtesy of Dr. O. Kamm of Parke Davis & Co. The Follutein was supplied through the courtesy of Dr. J. A. Morrell of E. R. Squibb & Son.

The pituitary glands of both beef and sheep were used for making the extracts prepared in this laboratory. The glands were received in a semi-frozen condition within a few days after collection at the abattoir. The anterior lobes of the beef pituitaries were dissected from the rest of the glands. The sheep pituitaries were trimmed of extraneous tissue and the entire glands used in the extraction work. The alkaline extractive method of Teel as modified by Bugbee, Simond, and Grimes (1931) yielded extracts rich in lactation-inducing activity but contaminated with the gonad-stimulating hormone. The growth hormone was inactivated by the preservative tricresol. During the course of this investigation a method for the preparation of the lactation factor (prolactin) free of the growth and gonad-

stimulating hormones was reported by Riddle, Bates, and Dykshorn (1933), but was unavailable for the experiments under discussion.

In the dosages employed no significant differences in the lactation-inducing activities of beef and sheep extracts were noted. With regard to time-stability, it is clearly indicated that little or no loss in activity occurs in extracts prepared from glands which have been kept frozen for as long as 6 months before being extracted. In extract form the lactation-inducing hormone remains stabile for at least 3 months, and is present in limited amounts 6 months after the date of preparation. There is evidence that extracts prepared from acetone-desiccated glands are not as active as those prepared from fresh or frozen glandular material.

The rabbit was chosen as the most suitable form for testing purposes. Ovulation occurs only after coitus in this species, and mature females kept in strict isolation and on an adequate dietary régime remain in a state of almost continuous oestrus, thus obviating the necessity of taking vaginal smears as must be done in the cases of spontaneously-ovulating animals like the rat, mouse, and guinea pig. A diet of egg mash and scratch feed mixed in equal proportions and slightly moistened along with generous amounts of alfalfa hay maintained the rabbits in excellent condition.

A routine procedure with regard to dosage, mode of administration, and the type of rabbit employed, was followed. Extract was administered subcutaneously after being warmed to body temperature and usually at a rate of 1 cc. of extract per kilogram of body-weight daily for a period of from 8 to 10 days. Injections were initiated only after it was made certain that the mammary glands were completely devoid of milk. Testing was conducted from the standpoint of the lactation response. In the following tables an "excellent" response is one in which the milk is expressible in copious streams and the mammary glands thickened as they are normally seen at the end of gestation. A "good" test is one in which the milk is expressible in thin spurts and the mammary glands slightly thickened. A "fair" test is one in which the milk is expressible in drops; the mammary glands are not noticeably thickened. In a test designated "scanty" a few small drops of milk are expressible and the mammary glands show no hypertrophy. Several preliminary tests

in which the character of the lactation responses of mature, ovariectomized females was compared with the responses of mature, intact females gave convincing evidence that the milk-yield was for all practical purposes the same in both groups, despite the fact that the ovaries of the intact rabbits were highly luteinized by the action of the gonad-stimulating hormone contained in the extracts. It is generally agreed (Corner, 1930; Nelson and Pfiffner, 1931; and others) that the lactation factor, once present in the blood stream, is not influenced by ovarian mediation. Intact animals were used in the following experiments except where otherwise stated.

TABLE I

EFFECTS OF ANTERIOR PITUITARY EXTRACTS UPON NULLIPAROUS RABBITS

Rabbit No.	Daily Dose c.c./kilo.	No. Days Injected	Lactation Started	No. Days Lactated	Character of Lact.	Effect upon Ovaries
16	2	10	neg.	neg.	.....	few blood points
16	1	10	neg.	neg.	.....	few blood points
18	1	10	neg.	neg.	.....	none
18	1	12	neg.	neg.	.....	none
17	2	10	3d day	9	fair	corpora lutea
17	1	8	3d day	5	scanty	corpora lutea
11	1	15	4th day	21	scanty	corpora lutea
11	1	10	3d day	30	fair	not examined
10	1	15	4th day	45	fair	corpora lutea
14*	1	15	6th day	18	fair	.....

* Ovariectomized.
Weights of animals ranged from 2200 gms. to 3150 gms.

Six distinct groups of rabbits were used, namely: immature, nulliparous, multiparous, and pregnant females and immature and mature males, the purpose being to determine to what degree the action of the lactation factor was limited by the sexual development, particularly of the mammary glands, of the injected animals.

THE EFFECTS OF ANTERIOR PITUITARY EXTRACT UPON NULLIPAROUS RABBITS

The first series consisted of five nulliparae individually isolated from the time they were weaned. Laparotomies performed prior to injections showed normal ovaries containing ripe follicles but no corpora lutea. Table I indicates the results of injections of anterior

pituitary extract rich in the lactation-inducing and gonad-stimulating hormones. In those cases in which the same animal was used twice a sufficient time-period (about 8 weeks) was allowed to elapse between the first and second series of injections to allow the mammary glands to become completely inactive. Examination of the table indicates at once a complete lack of uniformity in the manner in which these animals reacted. In the three animals that lactated the amount of milk secreted as well as the size attained by the mammary glands did not remotely resemble the condition seen in late pregnancy. Lactation became very scanty within a few days after the last injections although a few small drops of milk were expressible daily for as long as 45 days in one case. The failure of the two negatively reacting animals (Nos. 16 and 18) to lactate is tentatively ascribed to some anomalous deviation from normal threshold values. The reaction of the ovariectomized animal compared favorably with the reactions of the positive cases in the intact group. The point is indicated here and corroborated by work discussed in later sections that the lactation response is limited by the developmental state of the mammary glands prior to injections; the lactation-inducing hormone is not capable of bringing about a condition in the mature virgin mammary gland comparable to that seen in late pregnancy.

EFFECTS OF ANTERIOR PITUITARY EXTRACT UPON MULTIPAROUS RABBITS

A series of multiparous rabbits were injected with extract in the dosages indicated in Table II. In multiparae the mammary glands regress considerably following normal lactation. However, the number of secretory lobules present at the end of the period of regression is considerably greater than the number found in the mammary glands of the mature virgin rabbit (Turner, 1932*a*). It would appear that the lactation-inducing hormone, acting upon the more highly and uniformly developed mammaries of this type of rabbit, should induce lactation responses characterized by uniformity as to milk yield, and of considerable intensity. Eight animals were employed in this series, all of which had delivered young at some previous time within a 6-month period prior to injections. The mammary glands

were completely dry at the time injections were started. Table II indicates the results obtained. The striking uniformity in the responses of these animals is immediately apparent. Lactation and mammary-gland hypertrophy became evident on the third or fourth days of injection, reaching a peak at the time the last injections were administered, at which time the appearance of the mammary glands in the majority of cases was difficult to distinguish from that seen at the end of pregnancy. Very soon after the last injections (2 or 3 days) regression of the mammary glands as well as a diminution of

TABLE II

EFFECTS OF ANTERIOR PITUITARY EXTRACTS UPON MULTIPAROUS RABBITS

Rabbit No.	Daily Dose c.c./kilo.	No. Days Injected	Lact. Started	No. Days Lactated	Character of Lact.	Effect upon the Ovaries
15....	1	10	3d day	23	excellent	many corpora lutea
8.....	2	8	3d day	17	excellent	many corpora lutea
30....	1	10	3d day	20	good	many corpora lutea
33....	1	10	3d day	20	excellent	many corpora lutea
32....	1	10	4th day	20	good	many corpora lutea
8*....	1	10	3d day	8	excellent	many corpora lutea
62....	1	10	3d day	10	fair	few small corpora lutea
36†...	1	5	4th day	3	scanty	few small corpora lutea

* Same animal used on two occasions (about 6 weeks intervening).

† Died on sixth day of a respiratory infection.

milk flow set in. In those cases where the rabbits were not sacrificed following injections it was noted that secretory activity did not completely cease until about 25 days following the last injections. The ovaries in all cases were highly luteinized. The limited response of rabbit 62 (see Table II) was later found due to the partial potency of the extract employed upon that animal. Since a condition closely resembling that seen in late pregnancy may be brought about at will in the mammary glands of multiparous rabbits by means of anterior pituitary extracts, but not in nulliparous rabbits in which only a limited amount of milk secretion may be induced, it appears that at least one previous pregnancy is necessary to cause the lobules of the mammary glands to reach a maximum and uniform state of development. Turner and Frank (1931) have shown that injections of suitable amounts of theelin combined with the corpus luteum hormone (progestin) into castrate male rabbits induce a striking development

of both the ducts and lobules of the mammary glands, which is not brought about by injections of either of these two hormones when administered separately. It is believed that the previous action of both these hormones on the mammary glands of multiparous rabbits is the reason for this type of animals' mammary glands becoming fully active upon the injections of anterior pituitary extract. It is evident, however, that the initiation of lactation by means of anterior pituitary extract does not depend upon the corpus luteum hormone as indicated by the results obtained with ovariectomized nulliparous rabbits (Corner, 1930).

THE EFFECTS OF ANTERIOR PITUITARY EXTRACT UPON IMMATURE FEMALE RABBITS

The minimal age of puberty in the rabbit is given as $5\frac{1}{2}$ months by Hammond and Marshall (1925). It has been generally supposed that immature female rabbits will not lactate or show any mammary gland hypertrophy when injected with anterior pituitary extracts containing the lactation factor. (Asdell, 1931; Gardner, 1932). A series of immature female rabbits ranging in ages from 6 weeks to 4 months were studied. One animal, aged 6 weeks, was injected with 2 cc. of anterior pituitary extract, containing the lactation factor, daily for 10 days. No mammary growth or milk secretion were evident during that time. Autopsy revealed the ovaries, uterus, and mammary glands to be unaffected. A second rabbit, litter-mate to the first, was injected with 50 RU of theelin daily for 15 days. During that time no noticeable mammary gland growth occurred. Then 2 cc. of anterior pituitary extract along with 50 RU of theelin were injected daily for 11 days. During that time a slight reddening of the nipples was the only noticeable change occurring in the mammary glands. Autopsy performed the day after the last injections showed the mammaries to be unaffected, it being practically impossible to find any glandular tissue around the nipples. The ovaries were likewise unchanged, measuring slightly more than half a centimeter in length and showing smooth surfaces. The uterus was enlarged and highly vascularized, indicating the theelin effect. An intact female, aged 3 months and 2 weeks, was injected with 2.5 cc. of anterior pituitary extract daily for 11 days. At the beginning of the injection

period the mammary glands were in a completely rudimentary condition. On the third day the nipples were noticeably larger and a few small drops of clear fluid were expressible. Three days later the mammary glands were palpable but no milk could be expressed. Autopsy on the twelfth day showed the ovaries to be greatly enlarged and covered with corpora lutea. The mammary glands were spread considerably ($2\frac{1}{2}$ cm. from the bases of the nipples), but were devoid of milk. An intact female, aged 4 months, was injected with 1.5 cc. of anterior pituitary extract daily for 10 days. By the third day enlargement of the nipples was noted and from the fifth to the eleventh days a small quantity of milk was expressible from each teat daily. Autopsy on the twelfth day showed enlarged ovaries covered with corpora lutea and hemorrhagic follicles, and the mammary glands spread $3\frac{1}{2}$ cm. from the bases of the nipples. The main ducts were white with milk, and alveoli were present in abundance. From this it would appear that female rabbits not younger than 3 months are likely to respond to the gonad-stimulating and lactation hormones, though in a manner unsuitable for purposes of assay, owing to the very limited amount of milk secretion elicited.

EFFECTS OF ANTERIOR PITUITARY EXTRACT UPON MALE RABBITS

Nelson and Pfiffner (1931) were able to induce mammary gland growth and lactation in male rabbits and guinea pigs by injecting a corpus luteum extract in conjunction with anterior pituitary extract. Theelin alone, in the dosage employed, was found to be incapable of developing the mammary glands sufficiently to allow subsequent injections of anterior pituitary extract to induce lactation. Since it has been demonstrated that the preliminary action of the corpus luteum hormone is unnecessary in order to induce lactation in virgin female rabbits (Corner, 1930) a small series of males were injected with relatively huge amounts of theelin in the hope that the mammary glands would develop sufficiently to respond to subsequent injections of anterior pituitary extract. Two mature and two immature male rabbits were injected with 100 RU of theelin daily for 15 days followed by injections of 1 cc. of anterior pituitary extract per kilo daily for 10 days. Under such treatment the mammary glands de-

veloped slightly but failed to secrete milk during the injections of anterior pituitary extract. Nelson (1933) states that lutein-free theelin alone, if injected in pyramided doses over an 11-week period, will develop the guinea pig mammary gland to a size where it will secrete milk upon the injections of anterior pituitary extract. It has been observed, however, that theelin reacts much more readily upon the guinea pig mammary gland than upon the mammary glands of the rabbit. (Turner, 1932*b*; Smelser, 1933).

TABLE III

EFFECTS OF ANTERIOR PITUITARY EXTRACTS UPON PREGNANT RABBITS

Rabbit No.	Days Preg.	Dosage c.c./kilo	No. of Days	Lact. Started	Effect on Pregnancy	Remarks
25....	14	1	6	4th day	died 7th day	mammaries fully developed; milk in quantity
24....	15	1	7	3d day	died 8th day	mammaries fully developed; milk in quantity
26....	14	2.8	*	5th day	resorbed foetuses	mammaries extended but not thickened. milk in main ducts
35....	5	1	8	3d day	none	see text

* Extract given on fourteenth and twentieth days of pregnancy only.

EFFECTS OF ANTERIOR PITUITARY EXTRACT UPON PREGNANT RABBITS

In normal pregnancy there obviously exists some mechanism which inhibits milk secretion until parturition but which allows for the complete structural development of the mammary glands. Nelson (1932) offers evidence to show that this inhibitory influence is traceable to oestrin which acts as a suppressive influence upon the anterior hypophysis during pregnancy, thus inhibiting the release of the lactation factor until parturition. The following experiments were carried out to see if anterior pituitary extracts containing the lactation factor were capable of overriding this inhibitory mechanism and causing the premature secretion of milk in the pregnant animal. The findings are summarized in Table III. Injections (with one exception) were begun when the rabbits were in mid-pregnancy (the fourteenth or fifteenth days). Lactation started from 3 to 5 days following the first injections, but pregnancy was terminated; indeed, the treatment in two cases resulted in the deaths of the

mothers where standard dosages of extract was given. Using a smaller dosage, lactation occurred along with the resorbtion of the foetuses, but with no deleterious effects upon the mothers. These results show clearly that the lactation factor, once liberated in the bloodstream, is capable of causing the secretion of milk even in the presence of a mechanism which is "set," so to speak, to prevent its normal occurrence. One rabbit (No. 35) was injected on the fifth day of pregnancy with an extract known to be potent in only a slight degree for both the lactation-inducing and gonad-stimulating hormones. She was injected with 3 c.c. of extract daily for 8 days. On the third day scanty lactation was noted, which continued for 5 days, at which time autopsy was performed. The mammary glands were extended to the size normally seen in mid-pregnancy. Little alveolar thickening was noted but milk was present in the main ducts. The foetuses appeared normal. The ovaries showed two hemorrhagic follicles but were otherwise normal for the pregnant animal. From this it would appear that neither the ovarian or placental influences operative during pregnancy have any direct inhibitory effect upon the lactation-inducing hormone, but very likely, as suggested by Nelson (1932), operate through the anterior hypophysis. The importance of the placenta with regard to normal lactation is emphasized by recent investigations of Selye (1934). While Caesarian section initiates lactation in the rat he notes that if the uterus is filled with paraffin immediately following the removal of the foetuses lactation is inhibited, the corpora lutea persist, oestrus fails to follow and the mammary glands regress completely. Complete hysterectomy, however, when performed on the day of delivery, fails to interfere with lactation. Previously Nelson (1933) found that injections of anterior pituitary extract into pregnant guinea pigs induced lactation but terminated pregnancy. Normal lactation, however, depended upon the complete expulsion of the placenta. An article by Nelson (1934) appeared as this paper was being completed in which he states that the injection of anterior pituitary extract containing the lactation-inducing hormone into pregnant guinea pigs caused abortions followed by lactation. Since in no instance did lactation occur previous to abortion there was no direct evidence to indicate the possible induction of lactation by the introduction of the pituitary hormone. In the experiments on pregnant rabbits cited above, abortions did not

occur and lactation was initiated by means of pituitary extract with the foetuses in utero. This furnishes evidence that normally the non-occurrence of lactation during pregnancy is due to the absence of the lactation-inducing principle from the bloodstream and not to some inhibitory influence acting directly upon the mammary glands. The exact mechanism by which the lactation factor is liberated from the anterior hypophysis at parturition is not yet clear. It is indicated that a placental–ovarian-pituitary relationship (doubtless quantitative) is involved.

EFFECTS OF PREGNANCY URINE, FULLUTEIN, AND ANTUITRIN S UPON MULTIPAROUS RABBITS

Ascheim and Zondek (1928) were first to demonstrate that the urine of pregnant women contains, in addition to large amounts of oestrin, a substance which, when injected into immature female mice, causes ovulation and luteinization of the ovarian follicles. The effect is to all appearances identical to that produced by the gonad-stimulating hormone of the anterior pituitary. The following experiments were carried out to determine whether or not the anterior-pituitary-like principle of pregnancy urine has any lactation-inducing properties. Human pregnancy urine or extracts of it (Follutein, Antuitrin S) were injected in the following dosages and with the following results: Three multiparous rabbits were each injected twice daily with 10 cc. of pregnancy urine for a period of 10 days. Fresh samples of night urine were obtained from a normal 8-months-pregnant woman for this purpose. Injections were made into the ear veins. During the injection period no lactation occurred. Autopsies on the twelfth day revealed highly luteinized ovaries and the mammary glands developed to the condition seen in late pseudo-pregnancy. Two multiparous rabbits were each injected with 3 cc. of Follutein daily for 10 days. During that period no lactation occurred. Autopsies on the eleventh day revealed highly luteinized ovaries and mammary glands developed to the condition seen in late pseudo-pregnancy. An immature female rabbit, aged 4 months, injected with 1 cc. of Follutein daily for 8 days failed to lactate but revealed luteinized ovaries and well-extended mammary glands at autopsy. A multiparous rabbit was injected with 5 cc. of Antuitrin S daily for 8 days without lactating. Autopsy revealed similar condi-

tions in the ovaries and mammary glands to those mentioned above. An immature rabbit, aged 4 months, was injected with 3 cc. of Antuitrin S daily for 8 days without lactating. Autopsy on the ninth day revealed luteinized ovaries and well-extended mammary glands. It appears quite certain that the lactation-inducing hormone is entirely absent from pregnancy urine and extracts of pregnancy urine, although the luteinizing reaction is quite as intense and has the same physiological effect upon the mammary glands as that obtained with extracts of the anterior pituitary. The objection of Lyons and Catchpole (1933) to the use of intact rabbits for purposes of bio-assay, on the grounds that the setting up of a pseudo-pregnant condition might result in lactation engendered from the animal's own pituitary, seems untenable in view of the above experiments. Moreover, the lactation response elicited by anterior pituitary extract in intact rabbits becomes manifest long before any spontaneous lactation due to the pseudo-pregnant state occurs.

THE RELATIONSHIP BETWEEN DEGENERATIVE CORPORA LUTEA AND LACTATION

Despite the fact that lactation may be induced by means of anterior pituitary extract in the rabbit (Corner, 1930) and the guinea pig (Nelson and Smelser, 1933), in the absence of lutein influence the possibility of lutein involvement in the control of normal lactation is not materially lessened. It is well known that the termination of pseudo-pregnancy in the rabbit is accompanied by regression of the corpora lutea and the (at least temporary) secretion of milk. The fact that injections of human pregnancy urine or extracts of human pregnancy urine result in luteinization of the ovaries without causing lactation offered a ready means of establishing a luteal (pseudo-pregnant) phase without being complicated by lactation. It was thus possible to determine whether or not the loss of luteal function was concerned in the initiation of lactation. Luteal phases were established in four intact multiparous rabbits by injecting suitable doses of pregnancy urine, Follutein, or Antuitrin S. Laparotomies performed the day following the final injections showed the ovaries of all four animals to be highly luteinized. In all cases hypertrophy of the mammary glands began with the formation of the corpora lutea and spontaneous lactation occurred from 18 to 25 days later. Autopsies

performed several days after the onset of lactation revealed degenerate corpora lutea in the ovaries of all four rabbits (histological examination). In two cases the mammary glands were in the condition normally found in late pregnancy. In the other two cases the mammaries were not quite so well developed nor was the secretion of milk as intense as is found in late pregnancy. From these data a close relationship between lactation and loss of luteal function in the rabbit is indicated. Selye, Collip, and Thompson (1933) induced lactation in rats by essentially the same procedure. The injections of A.P.L. hormone of pregnancy urine caused luteinization of the ovaries and considerable mammary gland development but did not induce lactation. Removal of the luteinized ovaries resulted in lactation within 36 hours. Nelson (1932) found that extirpation of the non-luteinized ovaries of the virgin guinea pig resulted in lactation, and attributes this to the removal of oestrin inhibition on the anterior pituitary. Ovariectomy in the mature, virgin rabbit is not followed by lactation, judging from numerous cases under the writer's observation.

DISCUSSION

Comparative studies of the lactation responses of rabbits of both sexes and in various stages of sexual development to anterior pituitary extracts containing the lactation-inducing hormone indicate that the intact, multiparous rabbit is most suitable for the bio-assay of the lactation hormone. With the exception of the pregnant condition the mammary glands of this type of rabbit are the most highly and uniformly developed, and therefore the most reactive to the lactation stimulus. The use of intact animals has distinct advantages. From the standpoint of convenience the non-necessity of ovariectomy is readily appreciated. In work involving the purification of the lactation factor the reaction of the ovaries to the gonadotropic hormone provides a qualitative test of its presence in a given extract. The presence of functional corpora lutea (as judged by the development of the mammary glands to the pseudo-pregnant condition) brought about by injections of human pregnancy urine or extracts of human pregnancy urine does not set up a possible ovarian-pituitary-mammary gland relationship that would favor the release of the lactation-inducing hormone from the animal's own pituitary. The fact that lactation may be elicited experimentally in

ovariectomized rabbits clearly indicates the non-necessity of (at least immediate) ovarian mediation. Although lactation occurs coincident with corpus luteum degeneration, this phenomenon is far enough removed in time from the lactation induced by means of injections of anterior pituitary extract to offer no confusion in interpretation. Other considerations favor the use of the rabbit for assay purposes. Riddle, Bates, and Dykshorn (1933) were unable to induce lactation either in non-castrate or pregnant guinea pigs with prolactin and in the case of the latter with prolactin combined with the growth and gonadotropic hormones. In their hands the mouse proved to be unsatisfactory in its reaction to the lactation-inducing hormone. Nelson and Pfiffner (1931) were unable to induce lactation in the ovariectomized rat with anterior pituitary extract owing to the rapid regression of the mammary glands following ovariectomy. This would obviously preclude the possibility of comparing the responses of intact with ovariectomized rats. The further observation of Nelson (1932) that ovariectomy in the mature female guinea pig is followed by spontaneous lactation renders this species less suitable for assaying the lactation factor. It would appear that the ring-dove test of Riddle or the use of the multiparous rabbit as described offer the possibility of making assays uncomplicated by unfavorable species characteristics.

SUMMARY

1. The reactions of various (with regard to sexual development) types of rabbits to the lactation-inducing factor have been studied.

2. For the bio-assay of the lactation-inducing factor in the rabbit the intact, multipara has been found to be the most suitable.

3. The relation of the corpus luteum to the control of normal lactation is discussed.

LITERATURE CITED

ASCHEIM, S., and ZONDEK, B. 1928. Die Schwangerschaftdiagnose aus dem Harn durch Nachweis des Hypophysenvorderlappen Hormons. Klin. Wchnschr. **7**:1404.

ASDELL, S. A., 1931. Recent developments in the field of sex hormones. Cornell Vet. **21**:147.

BUGBEE, E. P.; SIMOND, A. E.; and GRIMES, H. M. 1931. Anterior pituitary hormones. Endocrinology. **15**:41.

CORNER, G. W. 1930. The hormonal control of lactation. I. Non-effect of the

corpus luteum. LL. Positive action of extracts of the hypophysis. Am. Jour. Physiol. **95**:43.

GARDNER, W. U. 1932. Sex and the internal secretions. Baltimore: Williams and Wilkins. P. 575.

HAMMOND, J., and MARSHALL, F. H. A. 1925. Reproduction in the rabbit. Edinburgh: Oliver and Boyd.

LYONS, W. R., and CATCHPOLE, H. R. 1933. Availability of the rabbit for assaying the hypophyseal lactogenic hormone. Proc. Soc. Exp. Biol. and Med. **31**:305.

NELSON, W. O., 1932. The reciprocal hypophyseal-ovarian relationship in the control of mammary-gland development and function. Anat. Rec. **54**:51 (supp.).

———. 1933. Reciprocal relationship between ovaries and anterior hypophysis as factor in control of lactation. Proc. Soc. Exp. Biol. and Med. **30**:953.

———. 1934. Studies on the physiology of lactation. III. The reciprocal hypophyseal-ovarian relationship as a factor in the control of lactation. Endocrinology. **18**:33.

NELSON, W. O., and PFIFFNER, J. J. 1931. Studies on the physiology of lactation. I. The relation of lactation to the ovarian and hypophyseal hormones. Anat. Rec. **51**:51.

NELSON, W. O., and SMELSER, G. K. 1933. Studies on the physiology of lactation. II. Lactation in the male guinea pig and its bearing on the corpus luteum problem. Am. Journ. Physiol. **103**:374.

RIDDLE, O.; BATES, R. W.; and DYKSHORN, S. W. 1933. The preparation, identification and assay of prolactin—a hormone of the anterior pituitary. Am. Journ. Physiol. **105**:191.

SELYE, H. 1934. Influence of uterus on mammary glands. Proc. Soc. Exp. Biol. and Med. **31**:488.

SELYE, H.; COLLIP, J. B.; and THOMPSON, D. L. 1933. Anterior pituitary and lactation. Proc. Soc. Expo. Biol. and Med. **30**:588.

SMELSER, G. K. 1933. The response of guinea pig mammary glands to injected sex hormones and ovarian grafts and its bearing on the problem of sex hormone antagonism. Physiol. Zoöl. **6**:403.

STRICKER, P., and GRUETER, F. 1929. Über die Wirkung eines Hypophysenvorderlappenhormons auf die Auslosung der Milchsekretion. Klin. Wchnschr. **8**:2322.

TURNER, C. W. 1932*a*, *b*. Sex and the internal secretions. Baltimore: Williams and Wilkins. Pp. 559, 567.

TURNER, C. W., and FRANK, 1931. The relation between oestrus-producing hormone and a corpus luteum extract on the growth of the mammary gland. Science. **73**:295.

TURNER, C. W., and GARDNER, W. U. 1931. The relation of the anterior pituitary hormones to the development and secretion of the mammary gland. Mo. Agr. Exp. Stat. Bull. **158**.

AN EXPERIMENTAL STUDY OF THE OPSONINS OF THE BLOOD. III. FURTHER STUDIES OF THEIR RELATIONSHIP TO ADRENAL CORTICAL FUNCTION[1]

(One figure)

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IN A previous report by the author (1931) evidence was presented to show that, following bilateral adrenalectomy in cats, a gradual and marked decrease occurred in the opsonic activity of the blood of such animals. The life-span of these operated cats averaged, approximately, $7\frac{1}{2}$ days, and during the terminal stages of adrenal insufficiency the opsonic activity of the blood serum had practically disappeared. That this decrease in activity was due to the lack of the adrenal cortical hormone rather than to non-specific metabolic changes in the animal occurring simultaneously with the onset of symptoms of cortical deficiency was indicated by the results of opsonic activity determinations made upon the blood of bilaterally nephrectomized animals. In such experiments no drop in opsonic activity occurred at any time following the removal of the second kidney.

With the isolation, in practically pure form, of the active principle of the adrenal cortex by Swingle and Pfiffner (1931) it became possible to study the effect of the administration of such extract upon the opsonic activity of the blood of normal and bilaterally adrenalectomized animals.

The results here reported give the findings of a series of experiments undertaken in an endeavor to throw further light upon the possible relationship between opsonic activity and adrenal cortical function.

MATERIALS AND METHODS

In the previously reported experiment (Blanchard, 1931) cats had been used exclusively because of the belief, then prevalent, but since shown to be erroneous, that rats, the animals most often used in re-

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sistance studies, were of little value as experimental animals because of the presence of unknown amounts of accessory cortical tissue. It has been shown (Pencharz, Olmsted, and Giragossintz, 1931; Gaunt, 1932, 1933) that, with proper operative technique, all the cortical tissue may be removed and that under such conditions rats would show the typical and fatal symptoms of adrenal cortical insufficiency.

In the present study both cats and rats were used. The opsonic activity of the blood of these animals was determined under the following conditions: before operation, that is, under normal conditions; after bilateral adrenalectomy, both with and without the administration of cortical extract; in normal animals receiving varying amounts of active extract.

The operative technique, in the case of cats, and the method of determination of opsonic activity, in all instances, was the same as previously reported (Blanchard, 1931). Adrenalectomy in the rat was performed in the manner outlined by Gaunt (1932, 1933).

The cortical extract was most kindly supplied by Dr. Swingle and represented some excess material sent to him by Parke, Davis & Co. for assay. This extract was of high potency, approximately 20 dog units per cc., and was, from a practical, physiological standpoint, adrenalin free.

EXPERIMENTAL

Opsonic activity of the blood of normal rats.—The opsonic activity of the blood of ten normal rats was determined. It was found that, by using large animals, sufficient blood could be obtained by cardiac puncture. Leucocytes were obtained, from rabbits, in the manner previously reported. Rabbit leucocytes react normally in ingesting foreign particles sensitized with rat serum. The results of this series of experiments are given in Table I. In this tabulation, as in all others following, the results are presented in terms of percentages of phagocytic cells.

Opsonic activity of the blood of bilaterally adrenalectomized rats.—The opsonic activity of the blood of seven bilaterally adrenalectomized rats was determined and showed that, following such operation, the same drop in opsonic activity occurred in rats as had been previously observed in cats. Because of the relative difficulty of ob-

taining from rats a sufficient quantity of blood at frequent intervals, terminal figures only were available.

TABLE I

OPSONIC ACTIVITY OF THE BLOOD OF NORMAL RATS

Animal	Serum Dilution (Ratio)						Spontaneous Phagocytosis
	Undiluted Serum	1:4	1:16	1:64	1:256	1:1,000	
51	93	76	53	41	30	22	18
52	86	74	63	44	31	20	18
53	90	80	66	45	29	21	18
54	88	67	52	44	31	23	16
55	86	64	50	38	25	19	16
56	90	77	64	51	36	22	16
57	87	69	47	36	24	18	16
58	88	74	57	40	28	20	15
59	84	68	47	35	22	18	15
60	91	77	63	45	31	23	15

A typical protocol follows:

Animal No. 1E. Rat, male. Operated 3/10/32. Both adrenal glands removed through two dorso-lateral incisions. Operations clean. No hemorrhage. Skin and muscle sutured with silk thread. Animal out of ether thirty minutes after operation. Two hours later appeared quite normal. Ate a little food.

3/11/32. Perfectly recovered and apparently normal. Ate usual amount. Active. No sign of bleeding or infection at the sites of incision.

3/12/32. Animal normal and lively. Wounds apparently healing cleanly.

3/13/32. Animal normal. 3/14/32–3/16/32. Animal normal. Eats regularly and in usual amount.

3/17/32. Animal somewhat weak. Ate about one-half usual amount.

3/18/32. Definite symptoms of adrenal insufficiency. Animal not active. Ate but small amount of food.

3/19/32. Very weak. Would not stand. Ate nothing. Rat bled at 2:30 P.M. by cardiac puncture. Two cc. blood taken. Opsonic activity of blood determined in the usual manner (see Table II).

3/20/32. Animal found dead in cage at 9:30 A.M. Condition indicated death about 2 hours earlier. Autopsy showed no recognizable adrenal cortical tissue at the site of the adrenal gland. No histological examination made. No infection. No signs of hemorrhage at the sites of operation nor at the site of cardiac puncture.

The results of this and of determinations upon the blood of six other bilaterally adrenalectomized rats showing acute symptoms of adrenal insufficiency are summarized in Table II. All results were controlled by simultaneous opsonic activity determinations of the blood of normal rats.

TABLE II

OPSONIC ACTIVITY OF THE BLOOD OF ADRENALECTOMIZED RATS DURING TERMINAL STAGES OF ADRENAL INSUFFICIENCY

Experimental rats marked "E"; control animals marked "C"

Animal	Date	Life-Span in Days	Serum Dilution (Ratio)						Spontaneous Phagocytosis
			Undiluted	1:4	1:16	1:64	1:256	1:1,000	
1E	3/19/32	10	42	31	25	22	24	20	17
1C	3/19/32	.....	94	78	55	42	30	24	
2E	4/2/32	4	33	28	20	18	21	21	19
2C	4/2/32	.....	85	73	60	45	32	22	
3E	4/3/32	5	30	22	14	19	15	17	16
3C	4/3/32	.....	90	80	67	46	30	22	
4E	4/14/32	10	24	17	20	18	21	17	18
4C	4/14/32	.....	87	68	50	43	32	23	
5E	4/20/32	6	33	21	15	16	18	15	14
5C	4/20/32	.....	88	64	51	39	26	19	
6E	4/20/32	8	30	20	13	15	12	14	14
6C	4/20/32	.....	88	64	51	39	26	19	
7E	4/22/32	4	31	18	12	15	16	15	16
7C	4/22/32	.....	85	62	50	37	25	17	

Opsonic activity of the blood of bilaterally adrenalectomized rats receiving injections of adrenal cortical extract.—Ten bilaterally adrenalectomized rats were treated with active cortical extract. All of these animals were allowed to show acute symptoms of adrenal insufficiency of such severity that, without treatment, death would have occurred within 12 to 18 hours. These animals were then injected with relatively large amounts of active cortical extract, and these injections were continued over a period of days until the rats showed complete recovery from the symptoms of cortical deficiency and were reacting in a normal manner. No blood was taken at the time of acute insufficiency, as the possibility of killing the animal by cardiac puncture at this time was sufficiently great to make this procedure seem inadvisable. From the data presented above it is safe

to assume that at this time the opsonic activity of the blood of these animals was at the usual low level. After the animals had completely recovered blood was drawn and the opsonic activity determined. At this time the animals were, by all possible criteria, perfectly normal, the extract having substituted for the removed cortical tissue.

Following this bleeding the extract was discontinued and the animals again allowed to show acute symptoms of deficiency. All of these rats died of adrenal insufficiency. From two such animals blood was taken at the time of terminal symptoms. The protocol of one follows:

Animal No. 28. Rat, female. Operated 4/15/32. Recovered normally from anesthetic and from operation.

4/16/32–4/19/32. Animal normal. Active. Ate well.

4/20/32. Rat rather weak. Ate about ½ usual amount.

4/21/32. Weak. Definite symptoms. Did not eat.

4/22/32. Animal prostrate. Not eating. Injected. 10:20 A.M. 4 cc. extract, subcutaneously. Injected. 5:00 P.M. 4 cc. extract, subcutaneously.

4/23/32. Animal much better. Still somewhat weak. Injected. 9:30 A.M. 4 cc. extract. Injected. 3:00 P.M. 2 cc. extract.

4/24/32. Animal much improved. By afternoon appeared perfectly normal. Ate well. Injected. 9:00 A.M. 2 cc. extract. Injected. 3:00 P.M. 2 cc. extract.

4/25/32. Rat apparently normal. Ate well. Lively. Bled at 2:30 P.M. by cardiac puncture. Recovered from bleeding. Injected. 5:30 P.M. 2 cc. extract. Opsonic activity of the blood determined (see Table III).

4/26/32. Animal normal. Injected. 4:00 P.M. 2 cc. extract.

4/27/32. Animal normal. Injections discontinued.

4/28/32. Animal normal. No injection.

4/29/32. Rat somewhat weak. Ate little. Definite early symptoms of adrenal insufficiency.

4/30/32. Animal prostrate. Definite terminal symptoms. Blood taken at 3:00 P.M. Opsonic activity determined. (See Table III.)

5/1/32. Animal found dead in cage. Autopsy showed normal abdominal viscera with no infection or hemorrhage.

The results of opsonic activity determinations in this series of animals are presented in Table III.

Opsonic activity of the blood of adrenalectomized cats receiving injections of adrenal cortical extract.—Since it was impossible to obtain from rats adequate amounts of blood at frequent intervals it was impossible to follow closely the changes in the opsonic activity of the blood of such animals during the period of onset of, at the time of,

and throughout the time of recovery from, a condition of acute adrenal insufficiency. In order to follow such changes a series of experiments were carried out upon cats. As has been shown in the first paper of this series (Blanchard, 1931), bilaterally adrenalectomized cats show a gradual decrease in the opsonic activity of their blood following the removal of the second adrenal gland.

TABLE III

OPSONIC ACTIVITY OF THE BLOOD OF FIVE ADRENALECTOMIZED RATS TREATED WITH ADRENAL CORTICAL EXTRACT

Animal No.	Date of Operation	Date of Acute Symptoms	Date When Bled	Number of Days of Extract	Condition at Time of Bleeding	Serum Dilution (Ratio)						Spontaneous Phagocytosis
						Undiluted	1:4	1:16	1:64	1:256	1:1,000	
26E	4/11/32	4/16/32	4/20/32	5	Normal	93	79	61	49	27	25	16
27E	4/11/32	4/15/32	4/20/32	6	Normal	87	76	65	48	35	21	.....
26–27C	.....	.....	4/20/32	.....	Normal	90	77	63	59	36	22	.....
28E	4/15/32	4/22/32	4/25/32	4	Normal	86	79	45	32	23	19	13
28C	.....	.....	4/25/32	.....	Normal	84	68	47	35	22	18	.....
$28E_2$	Acute symptoms for the second time. Bled 4/30/32. See protocol.				Prostrate	41	28	20	16	19	20	18
$28C_2$	.....	.....	4/30/32	.....	Normal	88	75	57	40	28	29	.....
29E	4/15/32	4/23/32	4/25/32	3	Normal	82	71	50	34	21	20	13
30E	4/18/32	4/22/32	4/25/32	4	Normal	80	67	50	27	21	19	.....
29–30C	.....	.....	4/25/32	.....	Normal	84	68	47	35	22	18	.....

Eight cats were used in this experiment. The operations were performed in the usual manner. All bilaterally operated animals were allowed to show acute symptoms of cortical deficiency and were then brought back to the normal condition by adequate injections of active cortical extract. This normal condition was maintained for some time by daily injections of extract. The injections were then discontinued and the animals again allowed to show acute symptoms of adrenal insufficiency. Several of the cats were again brought back to normal (see Fig. 1). During the entire course of these experiments a close check was kept of the opsonic activity of the blood of the experimental animals.

A typical protocol follows:

Animal No. 34. Cat. Black and white. Female.

9/14/32. Right adrenal removed. Weight 2,120 gms. Recovered normally.

9/23/32. Left adrenal removed. Weight 2,140 gms. Came out of ether well. Seemed to recover from the operation in a normal manner.

9/24/32. Seemed perfectly normal. Ate well. No abnormalities.

9/25/32–9/29/32. Cat perfectly normal. Active. Ate well.

9/30/32. Animal apparently normal. Ate well. Bled at 3:15 P.M. Opsonic activity of blood determined (Determination No. 34AE).

10/1/32. Cat normal. Ate well.

10/2/32. Normal. Bled 2:30 P.M. (opsonic activity Determination No. 34BE).

10/3/32. Did not eat all food. No definite symptoms.

10/4/32. Ate a little. Slight symptoms of general weakness.

10/5/32. Ate little. Drowsy. Definite symptoms of adrenal insufficiency. Bled at 2:45 P.M. Opsonic activity determined (Determination No. 34CE).

10/6/32. Marked and easily recognizable symptoms. Ate nothing. Will not walk.

10/7/32. Very drowsy. Will not stand. Bled 1:30 P.M. (Determination No. 34DE). Injected 4:00 P.M. 8 cc. extract, intraperitoneally.

10/8/32. Weak. Ate a little. Definitely improved. Injected. 9:30 A.M. 8 cc. extract, intraperitoneally. Injected. 4:45 P.M. 4 cc. extract, intraperitoneally.

10/9/32. Much improved. Ate more than half food. Bled 12:45 P.M. (Determination No. 34EE). Injected 3:00 P.M. 3 cc. extract, subcutaneously.

10/10/32. OK. Ate well. Active. Apparently normal. Injected 4:00 P.M. 3 cc. extract, subcutaneously.

10/11/32. OK. Injected 4:30 P.M. 3 cc. extract, subcutaneously.

10/12/32. OK. Injected 4:00 P.M. 3 cc. extract, subcutaneously.

10/13/32. OK. Bled at 10:45 A.M. (Determination No. 34FE). Injected 4:45 P.M. 3 cc. extract, subcutaneously.

10/14/32. OK. Injected 3 cc. extract, subcutaneously.

10/15/32. OK. Bled 12:30 P.M. (Determination No. 34GE). Injected 3 cc. extract, subcutaneously.

10/16/32. Cat perfectly normal. Injections discontinued.

10/17/32 and 10/18/32. Cat normal. No extract given.

10/19/32. OK. Bled 1:45 P.M. (Determination No. 34HE). No extract.

10/20/32. Slight weakness. Ate about $\frac{3}{4}$ food. No extract.

10/21/32. Definite symptoms. Ate little. Bled 2:40 P.M. (Determination No. 34IE).

10/22/32. Prostrate. Did not eat. Injected 3:00 P.M. 8 cc. extract, intraperitoneally.

10/23/32. Very weak. Ate little. Bled 1:35 P.M. (Determination No. 34JE). Injected 9:00 A.M. 8 cc. extract, intraperitoneally. Injected 5:00 P.M. 8 cc. extract, intraperitoneally.

10/24/32. Seems much better. Ate full meal. Injected 3 cc. subcutaneously at 2:30 P.M.

10/25/32. Apparently normal. Ate OK. Injected 3 cc., subcutaneously.

10/26/32. OK. Ate well. Bled 2:15 P.M. (Determination No. 34KE). Injected 3 cc. extract, subcutaneously.

10/27/32. OK. Injected 2 cc. extract, subcutaneously.

10/28/32. OK. Bled 12:15 P.M. (Determination No. 34LE). Injected 2 cc. extract, subcutaneously.

10/29/32–11/3/32. Cat OK. Active. Injected daily with 2 cc. extract, subcutaneously.

11/4/32. Cat OK. Extract discontinued. Bled 1:00 P.M. (Determination No. 34ME).

11/5/32–11/10/32. No extract given. Animal apparently normal.

11/11/32. Slightly weak. Did not eat well. No injection. Bled 11:30 A.M. (Determination No. 34NE).

11/12/32. Definite, subacute symptoms. No extract.

11/13/32. Marked symptoms. Quite weak. Bled 2:00 P.M. (Determination No. 34OE).

11/14/32. Very weak. Will not stand. No extract given.

11/15/32. Animal prostrate. Semi-conscious. Bled at 10:30 A.M. (Determination No. 34PE). Animal killed at 5:00 P.M. Autopsy showed perfectly normal abdominal picture. No evidence of infection or hemorrhage.

The results of the determination of the opsonic activity of cats No. 31 and No. 34 are summarized in Table IV and in Figure 1.

In Figure 1 an endeavor was made to show, in graphic form, the changes in the level of opsonic activity as related to time following the operation, to general metabolic and symptomatic conditions, and to periods of extract administration. This figure, for the sake of clarity, represents the determinations of but two experimental animals.

Opsonic activity of the blood of normal rats receiving daily injections of adrenal cortical extract.—It has been indicated by Hartman (1931) and by Hartman and Scott (1932) that adrenalectomized animals show increased resistance to colds and to bacterial infections following treatment with cortical hormone. It seems worth while, because of the reports of these investigators and the results reported above, to test the effect of daily injections of cortical extract upon the opsonic activity of normal rats.

Twenty rats were used in this experiment. Ten of the rats received subcutaneous injections of 2 cc. of cortical extract daily for a period of 10 days. The other ten rats received the same amount of

extract daily for 20 days. At the end of these periods the rats were bled and the opsonic activity of their blood determined. In each series of determination six normal, untreated animals were used as controls. By the use of this large number of controls in a single de-

TABLE IV

OPSONIC ACTIVITY OF THE BLOOD OF TWO BILATERALLY ADRENALECTOMIZED CATS RECEIVING ADRENAL CORTICAL EXTRACT

Cat No. 31. Female. Second operation 9/23/32. Bled at times indicated below. See also Figure 1.

Determination Number	Date	Condition of Animal	Serum Dilution (Ratio)						Spontaneous Phagocytosis
			Undiluted Serum	1:4	1:16	1:64	1:256	1:1,000	
31AE	10/1/32	weak	43	34	28	21	17	14	13
31AC	10/1/32	.....	85	71	57	42	28	15	.....
31BE	10/3	very weak	28	20	14	15	13	16	11
31BC	10/3	.....	91	73	59	41	30	22	.....
31CE	10/5	better	30	24	19	17	13	15	15
31CC	10/5	.....	87	70	59	43	29	22	.....
31DE	10/7	much better	62	49	40	32	24	15	11
31DC	10/7	.....	84	74	56	39	26	19	.....
31EE	10/9	normal	85	73	60	51	34	27	17
31EC	10/9	.....	88	72	61	48	35	26	.....
31FE	10/19	little weaker	41	30	23	15	11	12	14
31FC	10/19	.....	86	72	63	51	39	23	.....
31GE	10/21	prostrate	23	14	17	15	16	12	15
31GC	10/21	.....	90	77	64	52	38	24	.....
31HE	10/23	better	28	20	15	12	14	15	13
31HC	10/23	.....	92	80	68	54	40	28	.....
31IE	10/26	normal	80	64	49	35	24	18	15
31IC	10/26	.....	86	69	52	38	29	17	.....
31JE	10/28	seemed normal	79	61	53	35	27	14	12
31JC	10/28	.....	85	69	54	37	26	16	.....

Extract was discontinued on 10/27/32 and the cats died from adrenal insufficiency 11/8/33.

TABLE IV—*Continued*

Cat No. 34. Female. See protocol above. Second operation 9/23/32

Determination Number	Date	Condition of Animal	Serum Dilution (Ratio)						Spontaneous Phagocytosis
			Undiluted Serum	1:4	1:16	1:64	1:256	1:1,000	
34AE	9/30/32	seemed normal	63	50	39	28	20	18	13
34AC	9/30/32	.....	85	71	57	42	28	15	.....
34BE	10/2	seemed normal	51	39	30	22	17	12	11
34BC	10/2	.....	91	73	59	41	30	22	.....
34CE	10/5	drowsy	43	32	23	17	13	14	15
34CC	10/5	.....	87	70	59	43	29	22	.....
34DE	10/7	prostrate	16	12	14	10	13	15	11
34DC	10/7	.....	84	74	56	29	26	19	.....
34EE	10/9	better	32	25	15	16	14	17	17
34EC	10/9	.....	88	72	61	48	35	26	.....
34FE	10/13	normal	65	52	38	25	17	12	14
34FC	10/13	.....	84	70	58	43	28	19	.....
34GE	10/15	normal	91	77	63	49	33	24	12
34GC	10/15	.....	87	73	60	48	31	20	.....
34HE	10/19	seemed normal	75	61	48	33	27	18	14
34HC	10/19	.....	86	72	63	51	39	23	.....
34IE	10/21	weak	59	48	33	27	21	18	15
34IC	10/21	.....	89	77	64	52	38	24	.....
34JE	10/23	very weak	31	20	12	14	15	10	13
34JC	10/23	.....	92	80	68	54	40	28	.....
34KE	10/26	normal	62	47	39	24	20	13	15
34KC	10/26	.....	86	69	52	38	29	17	.....
34LE	10/28	normal	73	60	47	32	22	19	12
34LC	10/28	.....	85	69	54	37	26	16	.....
34ME	11/4	normal	87	73	59	46	34	23	17
34MC	11/4	.....	89	72	61	48	33	24	.....
34NE	11/11	weak	43	36	28	23	15	12	14
34NC	11/11	.....	84	70	57	43	30	21	.....
34OE	11/13	very weak	29	22	15	17	12	13	16
34OC	11/13	.....	85	71	55	40	34	22	.....
34PE	11/15	prostrate	19	12	11	14	12	13	11
34PC	11/15	.....	86	70	58	43	29	19	.....

Cat died 11/15/32 at 5:00 P.M.

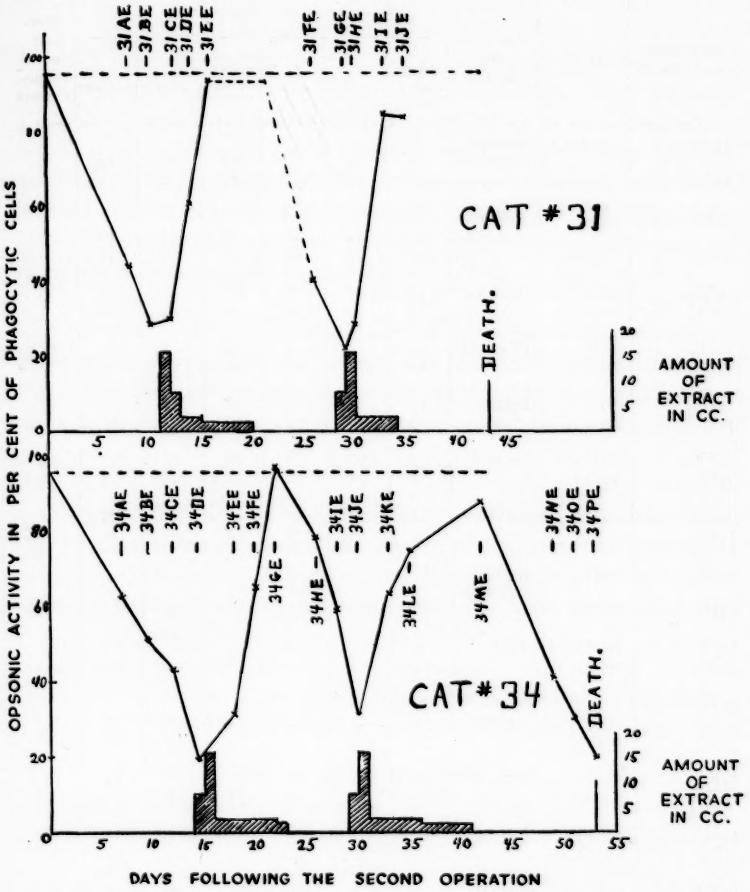

FIG. 1.—Numbers (31AE, 31BE, 34AE, etc.) refer to opsonic activity determinations as shown in Table IV and in the protocol of Cat No. 34. Shaded areas show days upon which extract was administered and amounts given on each day.

In plotting the curves all data are calculated to a common standard so that all determinations are comparable.

termination allowance was made for any slight variation in the opsonic activity of the blood of these normal rats.

The results of these two series of experiments are given in Table V.

From these results it seemed that daily injections of active cortical extract resulted in a slight increase above the normal of the opsonic activity of the blood. Those animals receiving extract for a period of 10 days showed an average increase in opsonic activity of about 6 per cent. Those receiving injections for a period of 20 days showed an average increase in opsonic activity of about 9 per cent.

This particular question is being studied in more detail at the present time.

Opsonic activity of active adrenal cortical extract.—Early in this study there was recognized the remote possibility that the adrenal cortical hormone might, itself, show opsonic activity. To check this possibility opsonic activity determinations were made on serial dilutions of cortical extract, with negative results. Also, varying amounts of adrenal cortical extract were added to serum from animals suffering from acute symptoms of adrenal insufficiency. This treatment had no effect in raising the opsonic activity from its low level. Cortical extract was added to samples of normal blood serum the opsonin of which had been destroyed by heating at a temperature of 60° C. for 1½ hours. No opsonic activity was observed following such treatment. It seems safe to assume, therefore, that, in all probability, the adrenal cortical hormone was not be be identified with "opsonin" nor did it show any opsonic activity.

DISCUSSION AND CONCLUSIONS

The results presented above indicate that bilaterally adrenalectomized rats show the same drop in opsonic activity of the blood as was previously observed in cats (Blanchard, 1931).

The data also show that the maintenance of normal conditions in adrenalectomized animals by injections of adequate amounts of cortical extract will, at the same time, maintain the opsonic activity of the blood at its normal level, and that during the period of recovery from acute symptoms of adrenal insufficiency the opsonic activity rises from its low value to the normal figure.

In addition, injections of cortical extract into normal rats result,

TABLE V

OPSONIC ACTIVITY OF THE BLOOD OF NORMAL RATS RECEIVING DAILY INJECTIONS OF ADRENAL CORTICAL EXTRACT

Animal	Period of Treatment in Days	Date	Serum Dilution (Ratio)						Spontaneous Phagocytosis
			Undiluted Serum	1:4	1:16	1:64	1:256	1:1,000	
61	10	2/6/33	76	63	52	34	26	21	15
62	10	2/6/33	80	70	61	38	26	21	15
63	10	2/6/33	77	63	51	32	23	18	15
64	10	2/6/33	81	68	53	35	24	20	15
65	10	2/6/33	80	71	62	37	25	20	15
66	10	2/6/33	76	62	53	41	24	17	15
67	10	2/6/33	79	64	50	40	27	19	15
68	10	2/6/33	75	61	49	33	21	16	15
69	10	2/6/33	82	70	61	49	30	20	15
70	10	2/6/33	73	62	46	35	20	17	15
Control		2/6/33	73	61	47	33	22	18	15
Control		2/6/33	77	62	49	35	24	17	15
Control		2/6/33	69	60	45	34	23	16	15
Control		2/6/33	68	58	43	32	24	17	15
Control		2/6/33	78	63	51	37	26	20	15
Control		2/6/33	73	62	50	40	27	19	15

Average increase of opsonic activity of treated animals above that of the controls—approximately 6 per cent.

71	20	2/16/33	80	67	54	39	28	22	17
72	20	2/16/33	86	71	60	45	31	25	17
73	20	2/16/33	83	69	55	43	30	23	17
74	20	2/16/33	81	70	58	41	28	20	17
75	20	2/16/33	87	73	60	49	34	22	17
76	20	2/16/33	90	79	66	50	37	24	17
77	20	2/16/33	85	71	60	43	31	20	17
78	20	2/16/33	84	72	59	44	32	21	17
79	20	2/16/33	79	66	51	40	29	19	17
80	20	2/16/33	78	67	50	37	26	19	17
Control		2/16/33	76	63	50	37	26	19	17
Control		2/16/33	80	68	52	36	25	18	17
Control		2/16/33	72	60	48	33	20	16	17
Control		2/16/33	76	65	51	40	26	18	17
Control		2/16/33	75	60	50	38	24	19	17
Control		2/16/33	79	65	53	40	27	20	17

Average increase of opsonic activity of treated animals above that of the normal controls—approximately 9 per cent.

after a period of days, in a slight rise above the normal level of opsonic activity of the blood of animals so treated.

In the interpretation of these results the writer, as before, makes no attempt to offer any explanation for the phenomena of opsonic sensitization and of phagocytosis. The concern is only with the changes in opsonic activity as effected by various degrees of adrenal cortical efficiency.

What explanation can be offered, therefore, for the results as here recorded? Two possible interpretations may be suggested: First, that the adrenal cortical hormone has some specific, stimulating action upon those cells of the body which produce "opsonin." That without this stimulus, that is, in the absence of the hormone, no "opsonin" is formed and the opsonic activity of the blood gradually disappears. That following substitution therapy with active cortical extract the hormone is once more present in the blood and the opsonin is again produced, eventually regaining its normal concentration in the blood, as indicated by a return of the normal opsonic activity. Second, that, with the gradual onset of the symptoms associated with adrenal insufficiency, changes occur in the relative concentration of the blood constituents, particularly in the colloids, electrolytes, and those radicals which effect the blood pH. These changes may upset the physicochemical balance of the blood serum to such an extent that the opsonin, while present, may be unable to sensitize the leucocyte-antigen complex. Fenn (1921*a*, 1921*b*), Mudd, Lucke, McCutcheon, and Strumia (1929) have indicated that phagocytosis is very possibly the result of changes in the interfacial relationships between leucocyte and foreign particle. In a blood serum of markedly abnormal composition it is not impossible to suppose that "opsonin" is incapable of bringing about the proper relationships necessary for the promotion of phagocytosis.

In an endeavor to evaluate these two possible interpretations the two following facts must be considered: In bilaterally nephrectomized animals no drop in opsonic activity occurs in spite of a marked change in blood composition (Blanchard, 1931). From this fact it seems safe to assume that mere change in blood composition is not sufficient to alter the opsonic activity of the serum. The second fact of importance in this consideration is that daily injections of cortical

extract into normal animals raise the opsonic activity of the blood. This would be the result one would expect were the cortical hormone to have a specific, stimulating effect upon the "opsonin" producing cells. Injections of adrenal cortical extract into normal animals do not alter the composition of the blood in its essential constituents.

It is therefore the belief of the writer that one function of the adrenal cortex is to control, in a manner not yet completely understood, the opsonic activity of the blood. An endeavor is now being made to gather additional data upon the relationship of adrenal cortical function to this and other serological phenomena.

SUMMARY

The opsonic activity of the blood of rats and cats was determined under several physiological conditions.

Following bilateral adrenalectomy there was a gradual and marked drop in opsonic activity.

Following injections of adrenal cortical extract into bilaterally operated animals showing terminal symptoms of adrenal insufficiency the opsonic activity gradually returned to normal.

The opsonic activity of the blood of normal rats, which were treated with cortical extract, was higher than that in untreated control animals.

Reasons for the belief that the adrenal cortical hormone has a specific effect upon the opsonic activity of the blood are given in the text.

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LITERATURE CITED

BLANCHARD, E. W. 1931. An experimental study of the opsonins of the blood. I. Effect of bilateral adrenalectomy. Physiol. Zoöl., **4**:302.

FENN, W. O. 1921*a*. The phagocytosis of solid particles. I. Jour. Gen. Physiol., **3**:439.

———. 1921*b*. The phagocytosis of solid particles. II. *Ibid.*, **3**:465.

GAUNT, R. 1932. Survival period of bilaterally adrenalectomized rats. Proc. Soc. Exper. Biol. and Med., **29**:823.

———. 1933. Adrenalectomy in the rat. Amer. Jour. Physiol., **103**:494.

HARTMAN, F. A. 1931. Increased resistance to cold produced by cortin after adrenalectomy. Proc. Soc. Exper. Biol. and Med., **28**:478.

HARTMAN, F. A., and SCOTT, W. J. M. 1932. Protection of adrenalectomized animals against bacterial infections by extract of adrenal cortex. Jour. Exp. Med., **55**:63.

MUDD, S.; LUCKE, B.; MCCUTCHEON, M.; and STRUMIA, M. 1929. On the mechanism of opsonin and bacteriotropin action. *Ibid.*, **49**:779.

PENCHARZ, R. I.; OLMSTED, J. M. D.; and GIRAGOSSINTZ, G. 1931. The survival of rats after total and partial adrenalectomy and adrenal transplantation. Physiol. Zoöl., **4**:501.

SWINGLE, W. W., and PFIFFNER, J. J. 1931. Studies on the adrenal cortex. II. *Ibid.*, **96**:164.